

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
23 December 2004 (23.12.2004)

PCT

(10) International Publication Number  
**WO 2004/110359 A2**

(51) International Patent Classification<sup>7</sup>: **A61K**  
(21) International Application Number:  
PCT/US2004/015661

(22) International Filing Date: 17 May 2004 (17.05.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/471,300 16 May 2003 (16.05.2003) US  
60/471,236 16 May 2003 (16.05.2003) US  
60/471,239 16 May 2003 (16.05.2003) US  
60/474,372 29 May 2003 (29.05.2003) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FUSION PROTEINS FOR THE TREATMENT OF CNS

(57) Abstract: This disclosure relates to compositions capable of use in the treatment of spinal cord injuries and related disorders of the central nervous system (CNS), and in particular, compositions including proteoglycan degrading molecules and compositions capable of blocking and/or over coming the activity of neuronal growth inhibitory molecules, as well as fusion proteins which includes a proteoglycan degrading domain and a domain capable of blocking and or over coming the activity of neuronal growth inhibitory molecules.



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## **FUSION PROTEINS FOR TREATMENT OF CNS**

### **BACKGROUND AND SUMMARY**

**[0002]** Spinal cord injury (SCI) inflicts trauma to the cells and tissues of the central nervous system (CNS) and causes a severe and debilitating condition in the individual. Following SCI, limited regeneration of injured neurons results in permanent disability characterized by some loss of sensation, paralysis and autonomic dysfunction. One reason that neurons fail to regenerate is their inability to traverse the glial scar that develops following SCI. This glial scar contains extracellular matrix molecules including chondroitin sulfate proteoglycans (CSPGs). In vitro studies show that neurons fail to extend processes over CSPG

coated surfaces, while in vivo data correlate failure of regeneration with areas of CSPG expression. Within the adult central nervous system (CNS) myelin there are also several identified axon growth inhibitor compounds like myelin associated glycoprotein (MAG), OMgp, and Reticulon 4 or Nogo that have been shown to be inhibitory for the growth of neurons.

**[0003]** The proteoglycan degrading enzyme chondroitinase ABC type I (**SEQ ID NO: X**) has been used to enhance neuronal growth in a dorsal column lesion model of spinal cord injury. It has also been reported that treating a spinal chord injury with NOGO receptor antagonist promotes a certain limited degree of neuronal regeneration. It has further been reported that creating a NOGO knock out mouse resulted in certain limited and inconsistent degrees of neuronal regeneration following dorsal hemisection of the spinal cord.

**[0004]** Experimental treatments for injury to the CNS have utilized the application of chondroitinase to the extracellular space, however the enzyme digests CSPG in the extracellular matrix and not intracellular stores. Furthermore, diffusion of chondroitinase within the parenchyma the essential and distinctive tissue of an organ or an abnormal growth as distinguished from its supportive framework) or between anatomical compartments is limited. The limited access of drugs, imaging agents, and anesthetics, etc. to target cells and/or tissues of the Central Nervous System (CNS) may reduce the usefulness or effectiveness of any of these substances.

**[0005]** The delivery of theraprutic and diagnostic molecules into cells and tissues is, in part, dependent upon extracellular matrices as well as carbohydrates and proteins linked to cell membranes. The extracellular matrix is composed in part of proteoglycans, among them are the chondroitin sulfated proteoglycans (CSPGs). CSPGs are a family of proteoglycans composed of a core protein and covalently linked sulfated glycosaminoglycans. Each proteoglycan is

determined by the glycosaminoglycan side chains. For CSPGs these side chains are made up of approximately 40 to 100 sulfated disaccharides composed of chondroitin 4, 6 and dermatan sulfates. The protein component of the CSPG is ribosomally synthesized and the glycosylation occurs in the endoplasmic reticulum and Golgi apparatus. The sugar chains are then sulfated at the 4 or 6 positions by several glycosaminoglycan sulfotransferases.

**[0006]** Transduction proteins may be used to transport polypeptides and polynucleotides cargo across anatomical barriers and into cells. For example, the TAT protein from the human immunodeficiency virus (HIV) contains a protein transduction domain (PTD) that is involved in the transduction of HIV into cells. The PTD contains an 11 amino acid domain (TAT Peptide) that is responsible for the activity of the PTD. The TAT Peptide can be linked to proteins and facilitate the transduction of the proteins into cells. The mechanism of transduction is independent of the molecular weight or chemical properties of the proteins that are linked to the TAT Peptide. In vivo studies show that if a fusion protein consisting of the TAT Peptide linked to the 120 kd enzyme, beta-galactosidase ( $\beta$ -Gal), is injected into mice, then a robust delivery of  $\beta$ -Gal into a wide variety of cells is observed. When linked to the TAT transduction peptide,  $\beta$ -Gal activity was observed in the brain; without the TAT Peptide,  $\beta$ -Gal was not observed in the brain. Transport across the blood brain barrier is normally restricted to certain hydrophobic small molecules and particular low molecular weight lipophilic peptides. Transport of proteins as large as  $\beta$ -Gal into the brain is usually not possible without substantial disruption of the blood brain barrier, but the TAT Peptide facilitates transport while leaving the blood brain barrier intact.

**[0007]** Chimeric proteins, also called fusion proteins, are hybrid proteins which combine at least parts of two or more precursor proteins or polypeptides. Chimeric proteins may be



produced by recombinant technology, i.e. by fusing at least a part of the coding sequence of one gene to at least a part of the coding sequence of another gene. The fused gene may then be used to transform a suitable organism which then expresses the fusion protein.

[0008] Tat Peptide Complexes Frankel et al. (U.S. Pat. Nos. 5,804,604; 5,747,641; 5,674,980; 5,670,617; 5,652,122) discloses the use of Tat peptides to transport covalently linked biologically active cargo molecules into the cytoplasm and nuclei of cells. Frankel only discloses covalently linked cargo moieties that are (therapeutic, diagnostic or prophylactic), and does not teach or suggest the attachment of molecules that facilitate diffusion, plasticity, neurite growth, and axon regeneration. These molecules can include but are not limited to molecules that overcome neurite outgrowth inhibition, or promote nerve growth such as soluble NOGO antagonists like NgR<sub>27-311</sub>, neural cell adhesion molecules like L1, neurotrophic factors, growth factors, phosphodiesterase inhibitors, and inhibitors of MAG or MOG. Additionally, deletion mutants may be combined with other compounds that promote remyelination such as neuregulins (GGF2) and antibodies that promote remyelination. or proteoglycan degrading molecules to Tat peptides.

[0009] Regeneration following SCI is limited because of a variety of obstacles that include the deposition of CSPG at the glial scar, demyelination of axons, lack of trophic support and lack of axonal guidance. A single therapy directed against one aspect of SCI may not be as effective as a combinatorial approach. Fusion proteins with chondroitinase will allow combinatorial therapy with a single protein. Fusion partners for chondroitinase that will be constructed in this proposal were chosen from among proteins that have evidence for efficacy in SCI.

[0010] The use of a molecule that has the ability to both degrade extracellular matrix glycoproteins and to block or overcome the inhibitory nature of myelin components may be used to improve the ability of damaged neurons to grow or regenerate compared with either treatment alone. The proteoglycan degrading molecules may also be used advantageously to provide a method of facilitating access and diffusion of substances into cells or tissues through the use of at least one enzyme capable of cleaving proteoglycans and preferably degrading chondroitin sulfate proteoglycans (CSPG).

[0011] Embodiments of the present invention include compositions that comprise polypeptides which cleave proteoglycans, polypeptides that block and/or overcome the activity of neuronal growth inhibitory molecules, or a combination of these. The compositions containing the proteoglycan degrading molecule or neuronal growth inhibitory molecules may also include molecules for transduction of the polypeptides across cell membranes and the blood brain barrier. The compositions may be used in the treatment of spinal cord injuries and related disorders of the central nervous system (CNS). The compositions can be used in the regeneration of damaged neurological tissue and facilitate the diffusion and transport of therapeutic molecules capable of blocking and/or overcoming the activity of neuronal growth inhibitory molecules into damaged or diseased tissue.

[0012] Embodiments of the present invention include compositions and methods for their use to facilitate delivery and diffusion of therapeutics or diagnostic agents, and preferably agents that promote regeneration of nerves and axons, into cells or tissues. Preferably the composition includes the use of an enzyme capable of cleaving chondroitin sulfate proteoglycans (CSPG) to increase the diffusion of these agents into cells or tissues of the central nervous system.

**[0013]** Compositions of the present invention may include chimeric or fusion proteins capable of systemic use in the treatment of spinal cord injuries and related disorders of the central nervous system (CNS), and in particular, fusion proteins capable of crossing the blood brain barrier. The fusion protein may include a polypeptide transduction domain, a polypeptide domain capable of degrading a proteoglycan, preferably a domain cleaving chondroitin sulfate proteoglycan (CSPG), a polypeptide domain that blocks and or over comes the activity of neuronal growth inhibitory molecules, or any combination of these polypeptide domains that may be used in the treatment of spinal cord injuries and related disorders of the central nervous system (CNS) . The various polypeptide domains may be linked or chemically bonded together by polypeptide linkers.

**[0014]** Compositions of the present invention include polynucleotides which encode for the chimeric or fusion proteins capable of systemic use in the treatment of spinal cord injuries and related disorders of the central nervous system (CNS), and in particular, they encode for fusion proteins capable of crossing the blood brain barrier. The polynucleotides which encode for these chimeric or fusion proteins may include a polynucleotide domain encoding for a polypeptide transduction domain, a polynucleotide domain encoding for a polypeptide domain capable of degrading a proteoglycan, preferably cleaving chondroitin sulfate proteoglycan (CSPG), a polynucleotide domain encoding for a polypeptide domain that blocks and or over comes the activity of neuronal growth inhibitory molecules, or any combination of these domains that may be used in the treatment of spinal cord injuries and related disorders of the central nervous system (CNS) . The polynucleotide also includes one or more polynucleotide domains that encode for polypeptides that link the domains of the polypeptide together to form the fusion protein.

**[0015]** One embodiment of the present invention is a composition and a method for its use that facilitates the access and distribution of therapeutic and diagnostic agent in the composition into cells, through membranes or into tissues by the use of composition that includes at least one enzyme capable of cleaving proteoglycans, preferably the composition includes a fusion protein having an enzyme capable of cleaving CSPG's. The molecules or agents in the composition may include one or more of Growth factors including, Brain Derived Neurotrophic Factor, Insulin-like Growth Factor, Fibroblast Growth Factor, Ciliary Neurotrophic Factor, Glial Derived Neurotrophic Factor, Transforming Growth Factor, Glial Growth Factor 2, L1, GM1, Vascular Endothelial Growth Factor, Nerve Growth Factor, Immunophilins. Molecules in the composition can include fluorescent or contrast agents for imaging. The agents may include cells for transplant – stem cells, neurons, others, cells as delivery agents, chemotherapeutic agents, antibiotics, antibody therapies, Nogo receptor antagonists, other chondroitinase enzymes. The composition may include a transduction domain, an enzyme capable of cleaving proteoglycans, or both. Preferably the composition includes a fusion protein having a transduction domain, an enzyme domain capable of cleaving proteoglycans, or both. The fusion protein can facilitate the transport or modifies transport of such agents into cells, tissues, and/or otherwise inaccessible locations; and/or to enhance penetration rates, distance of penetration; or provide more even concentration distribution. Preferably the modified transport occurs through the use of at least one enzyme capable of cleaving CSPGs. The compositions can be used for treating a CNS injury, preferably the composition is used in the treatment of neuronal damage from a contusion injury.

**[0016]** Embodiments of the present invention include chimeric proteins of a proteoglycan degrading domain linked to a polypeptide that blocks the action of neuronal growth inhibitors

such as but not limited to a Nogo-receptor antagonist (NgR<sub>27-311</sub>) domain or variant linked to a chondroitinase like chondroitinase ABC I or a variant of chondroitinase having one or more N terminal amino acids deleted. The compound may include chimeric proteins of a proteoglycan degrading domain linked to a polypeptide that is a neural cell adhesion promoter such as an L1 neural cell adhesion promoter domain or variant linked to chondroitinase ABC I or a variant of chondroitinase having one or more N terminal amino acids deleted. The chimeric proteins may include chimeric proteins of a proteoglycan degrading domain linked to a polypeptide that is a glial cell stimulator, such as but not limited to a GGF2 glial cell stimulator or variant linked to chondroitinase ABCI or a variant of chondroitinase having one or more N terminal amino acids deleted.

[0017] An *E.Coli* recombinant expression system and purification process can be used to produce essentially pure and catalytically active chondroitinase ABCI. These methods may be modified for producing chimeras of proteoglycan degrading molecules and other agents.

[0018] The chimera may be assayed for chondroitinase enzymatic activity and the specific biological activity of each fusion partner. Methods to measure the activities of the chimera may be modified for those used to measure chondroitinase activity including a spectrophotometric assay, zymography, an HPLC assay to detect CSPG disaccharide digestion products and an in vitro neurite outgrowth assay. A neuron growth cone collapse assay can be used to evaluate NOGO receptor antagonists and a neurite outgrowth assay can be used measure LI activity. GGF2 activity may be measured using a Schwann cell proliferation assay.

[0019] The compositions and method of the present invention can be used for the treatment of spinal cord injuries and in the promotion of regeneration of axons. The compositions of the present invention can also be used to promote plasticity, regrowth, repair,

and/or regeneration of dysfunctional neurons in the CNS that have been damaged as a result of disease, such as degenerative diseases including Alzheimer's and Parkinson's disease.

Advantageously, the use of proteoglycan degrading polypeptides or membrane transducing polypeptides in the compositions of the present invention also promote diffusion and access of damage or diseased tissue to other therapeutic agents promoting the regeneration of neurons.

### DESCRIPTION OF THE DRAWINGS

**[0020]** The file of this patent contains at least one drawing/photograph executed in color. Copies of this patent with color drawing(s)/photograph(s) will be provided by the Office upon request and payment of the necessary fee.

**[0021]** In part, other aspects, features, benefits and advantages of the embodiments of the present invention will be apparent with regard to the following description, appended claims and accompanying drawings where:

**[0022]** FIG. 1 is an illustrative example of a TAT-chondroitinase ABCI construct; the DNA sequence for the entire gene fragment fused with the Tat sequence followed by the 5-glycine linker; the sense and antisense oligonucleotides having the sequences as 5'-tatgtatggcgtaaaaagcgtcgtcaacgtcgtcgtggtggtggtggtca-3' and 5'-tatgaccaccaccaccaccacgacgacgttgacgacgctttttacgaccataca-3' were annealed and ligated at the NdeI site which is at the 5' end of ABCI gene cloned in pET15b (Novagen) at the NdeI and BamHI sites. The Tat<sub>48-57</sub> peptide sequence (GRKKRRQRR) is joined with the ABCI sequence by a pentaglycine linker.

**[0023]** FIG. 2 are Brain section images; (I) illustrating lobes from adult rat which were incubated in beta-galactodidase alone (B&D), or with the addition of Chondroitinase ABCI (A, 0.5 U/ml or C, 0.005 U/ml); (II) Eosin Y penetration through the cortex of a Chondroitinase treated brain hemisphere and control showing approximately the same penetration, Eosin Y is a

is a zwitterionic, having an overall negative charge at the low pH it was used at, and it is 692 kDa; (III) A saturate solution of Congo Red demonstrates greater penetration through the cortex of a Chondroitinase treated brain hemisphere as compared to untreated brain, Congo red is a negatively charged dye of 697 kDa;

[0024] FIG. 3 (A) is a diagram representing the full length GGF protein (GGF2-M<sub>1</sub>-E<sub>422</sub>) and the three GGF2 fragments containing the Ig and EFG domains alone and in combination; (B) a schematic presentation of chimeric proteins of a proteoglycan degrading molecule like chondroitinase ABCI and a neuregulin 1 gene isoform GGF2;

[0025] FIG. 4 illustrate the structure of (A) an NgR<sub>27-311</sub>- N terminal chondroitinase ABCI chimeric protein with and without a peptide spacer or linking group; (B) an NgR<sub>27-311</sub>- C terminal chondroitinase ABCI chimeric protein with and without a peptide spacer or linking group; (C) an extracellular domain L1 N-terminal chondroitinase ABCI chimeric protein with and without a spacer or linking peptide; (D) an extracellular domain L1 C-terminal chondroitinase ABCI chimeric protein with and without a spacer or linking peptide.

[0026] FIG. 5 (A) BBB scores from spinal cord injured animals treated with chondroitinase (Chondroitinase ABC I), penicillinase or artificial cerebrospinal fluid (aCSF); (B) Parasagittal sections of spinal cords from injured animals treated with chondroitinase (left column) or penicillinase (right column). Sections were cut at 30 microns. Each set of images contains four parasagittal sections realigned with the rostral cord to the left and the caudal cord to the right. The most central section of each set has been removed and replaced below to aid visualization. Pairs of images are Weil stained, anti-GFAP and an amino cupric stain of neuronal degeneration (top to bottom). (C) and (D) Distribution of individual BBB scores according to

treatment group. Scores are BBB scores at ten weeks post surgery. Group averages are shown below the data points.

### **DETAILED DESCRIPTION**

**[0027]** Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

**[0028]** It must also be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated by reference. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

**[0029]** "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event occurs and instances where it does not.



**[0030]** Suitable enzymes that cleave CSPGs include chondroitinase ABC type I, chondroitinase ABC Type II, chondroitinase AC and Chondroitinase B or mammalian enzymes with chondroitinase-like activity such as hyaluronidase 1, hyaluronidase 2, hyaluronidase 3, hyaluronidase 4, cathepsins, ADAMTs and PH-20 or mixtures thereof.

**[0031]** CSPGs are a family of proteoglycans composed of a core protein and covalently linked sulfated glycosaminoglycans. The polysaccharide chains are cleaved by several enzymes, including a family of chondroitinases. To date, this enzyme family contains at least four members, Chondroitinase ABC I, ABC II, AC and B. Chondroitinase ABC I is an exo-lyase which cleaves both chondroitin and dermatan sulfates. It has been used extensively in the study of in vitro neuronal regeneration over CSPG-laden substrates and more recently in in vivo studies following CNS injury.

**[0032]** Proteins and polypeptide that may be used in the compositions and fusion proteins of the present invention are those which promote plasticity as well as the regeneration of injured or diseased neurons and axons. These regenerating proteins and polypeptides may include cell adhesion proteins, those which stimulate glial cells, and polypeptide which block the inhibitory effect of proteins that act as axon growth inhibitors.

**[0033]** Plasticity of the nervous system refers to any type of functional reorganization. This reorganization occurs with development, learning and memory and brain repair. The structural changes that occur with plasticity may include synapse formation, synapse removal, neurite sprouting and may even include strengthening or weakening existing synapses. Regeneration is generally differentiated from plasticity by the long range growth of axons in disrupted tracts that is characteristic of regeneration.

**[0034]** Proteins and polypeptides are capable of blocking the activity of neuronal growth inhibitory molecules may include peptides and polypeptides that block the inhibitory properties of peptide such as but not limited Nogo, MAG, OMgp. Suitable compositions that overcome the activity of neuronal growth inhibitory molecules include but are not limited to Protein Kinase C family inhibitors, Rho Kinase family inhibitors and agents, such as phosphodiesterase inhibitors, that increase intracellular cyclic AMP, and L1. The (NgR<sub>27-311</sub>) peptide has been shown to inhibit binding of Nogo66, OMgp, MAG, and MOG to membrane-bound NogoR and overcome the inhibitory effects of Nogo on the regeneration of nerve processes.

**[0035]** Proteins and polypeptides that affect cell adhesion or stimulate cells may include but are not limited to polypeptides such as L1 and GGF2. L1 is a neural cell adhesion protein that is a potent stimulator of neurite outgrowth in vitro for which has been found that treatment of acute SCI in rodents using a soluble form of L1 leads to an increase in the recovery of neurological function. GGF2 stimulates glial cells and has been shown to improve clinical outcome measures in a murine model of experimental allergic encephalomyelitis (EAE) likely as a result of the stimulation of oligodendrocytes to promote remyelination.

**[0036]** Cell membrane-permeant peptide sequences useful in practicing the present invention include, but are not limited to, RQARRNRRRRWRERQR-51 (HIV-1 Rev protein basic motif; (SEQ ID NO:51)); MPKTRRRPRRSQRKRPTP-119 (HTLV-1 Rex protein basic motif; SEQ ID NO:52)) (Kubota et al. 1989); the third helix of the homeodomain of Antennapedia (Derossi, et al., J. Biol. Chem. 271:18188-93, 1996) (43-RQLIWFQNRRMKWLL-58 (SEQ ID NO:53)); a peptide derivable from the heavy chain variable region of an anti-DNA monoclonal antibody (Avrameas, et al., Proc. Natl. Acad. Sci. 95:5601-06, 1998) (VAYISRGGVSTYYSDTVKGRFTRQKYNKRA (SEQ ID NO:54)); and the

Herpes simplex virus VP22 protein (Elliot and O'Hare, Cell, 88:223-33, 1997) (1-

MTSRRSVKSGPREVPRDEYEDLYYTPSSGMASPDSPPDTSRRGALQTRSRQR

GEVRFVQYDESDYALYGGSSSEDEHPEVPRTRRPVSGAVLSGPGPARAPPPP

AGSGGAGRTPTTAPRAPRTQRVATKAPAAPAAETTRGRKSAQPESAALPDAP

ASRAPTVQLWQMSRPRTDEDLNELLGITHRVTVCEGKNLLQRANELVNPDV

VQDVDAATATRGRSAASRPTERPRAPARSASRPRRPVE-246 (SEQ ID NO:55)). In a

preferred embodiment, the basic peptide is derivable from the human immunodeficiency virus

type 1 (HIV-1) Tat protein (Fawell et al., Proc. Natl. Acad. Sci., 91:664-68, 1994). In particular,

the Tat peptide can comprise any sequential residues of the Tat protein basic peptide motif 37-72

(Vives et al. 1997) (37-CFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQ-72 (SEQ ID

NO:56)). The minimum number of amino acid residues can be in the range of from about three to

about six, preferably from about three to about five, and most preferably about four, i.e., the

minimal requirement for one alpha helical turn. A preferred embodiment comprises Tat protein

residues 48-57 (GRKKRRQRRR) (SEQ ID NO:57).

**[0037]** Suitable PTD domains include those derived from the TAT protein. Tat Proteins and Peptides Tat is an 86-amino acid protein involved in the replication of human immunodeficiency virus type 1 (HIV-1). The HIV-1 Tat transactivation protein is efficiently taken up by cells (Mann and Frankel 1991; Vives et al. 1994), and low concentrations (nM) are sufficient to transactivate a reporter gene expressed from the HIV-1 promoter (Mann and Frankel 1991). Exogenous Tat protein is able to translocate through the plasma membrane and reach the nucleus to transactivate the viral genome.

**[0038]** A region of the Tat protein centered on a cluster of basic amino acids is believed to be responsible for this translocation activity (Vives et al. 1997). Tat peptide-mediated cellular

uptake and nuclear translocation have been demonstrated in several systems (Vives, et al., J Biol Chem 272:16010-16017, 1997; Jones, Genes Dev 11:2593-2599, 1997). Chemically coupling a Tat-derived peptide (residues 37-72) to several proteins results in their internalization in several cell lines or tissues (Fawell, et al., Proc Natl Acad Sci USA 91:664-668, 1994; Anderson, et al., Biochem Biophys Res Commun 194:876-8884, 1993; Fahraeus, et al., Curr Biol 6:84-91, 1996; Nagahara, et al., Nat Med 4:1449-1452, 1998). A synthetic peptide consisting of the Tat basic amino acids 48-60 with a cysteine residue at the C-terminus coupled to fluorescein maleimide translocates to the cell nucleus as determined by fluorescence microscopy (Vives et al. 1997). In addition, a fusion protein (Tat-NLS-.beta.-Gal) consisting of Tat amino acids 48-59 fused by their amino-terminus to .beta.-galactosidase amino acids 9-1023 translocates to the cell nucleus in an ATP-dependent, cytosolic factor-independent manner (Efthymiadis et al. 1998).

**[0039]** Chimeric proteins, also referred to in the art as fusion proteins, are hybrid proteins which combine at least parts of two or more precursor proteins or peptides. Chimeric proteins may be produced by recombinant technology, i.e. by fusing at least a part of the coding sequence of one gene to at least a part of the coding sequence of another gene. Where desirable, one or more genes for linker peptides may be fused to the coding sequence of genes for the other polypeptide domains in the fusion protein. The fused gene may then be used to transform a suitable organism such as but not limited to *E. coli* or CHO cells which then expresses the fusion protein.

**[0040]** Genes encoding either N or C terminal deletion mutants of polypeptide domains of the fusion proteins can be used in constructs for expression of the fusion proteins. Preferably the generated deletion mutants maintain their catalytic proteoglycan degrading activity, blocking activity, growth activity, or transduction activity. Generated deletion mutants of the

proteoglycan degrading molecules like chondroitinase ABCI enzyme where the mutant is missing a certain number of amino acids from the N and or C-terminal are those that retain some proteoglycan degrading activity. N-terminal deletions of chondroitinase like chondroitinase ABC I maintain a histidine-tag that is attached to the N-terminus. It is expected that a TAT-deletion mutant chondroitinase ABC I fusion DNA construct can be expressed without removal of the TAT polypeptide during expression. For example Fuse TAT peptide at the N-terminus of either ABCI-N $\Delta$ 20 or ABCI-N $\Delta$ 60 deletion mutant. Fragments of polypeptides, such as those shown in FIG. 3 for GGF2, may be used in the construction of chimeric fusion proteins.

**[0041]** Catalytically active deletion mutants of chondroitinase ABCI can be prepared for example but not limited to deleting 20, 40 and 60 amino acids respectively from the N-terminus of the mature ABCI protein. Deletion of 60 amino acids from the N terminal and 80 amino acids from the C-terminal end may also be used to make a deletion mutant of a proteoglycan degrading chondroitinase ABCI. These deletion mutants and those of other proteoglycan degrading molecules may used for construction of N-terminal fusion chimeric protein. Detailed comparative biochemical studies can be done to determine the efficacy of mature chondroitinase ABCI versus various deletion mutant in compositions and fusion proteins with respect to the substrate specificity, substrate binding and tissue penetration.

**[0042]** A mutant of chondroitinase ABCI that has native protein structure, but lacks catalytic activity may be prepared as a null or a negative control for bioassays and SCI studies. Based on the crystal structure of chondroitinase ABCI a site-specific mutant designated H501a and Y508a to knock out catalytic activity in the putative active site can be prepared. Such mutants can be tested for inactivation of catalytic activity and SEC to compare to the wild-type

enzyme. If the null activity mutant is successfully created it will provide a negative control for the various fusion proteins for use in bioassays and ultimately in SCI animal studies.

**[0043]** An *E. Coli* expression system may be to make chondroitinase using PET expression vectors (Novagen). The GGF2-chondroitinase ABCI fusion protein may be expressed in *E. coli*. Constructs for Tat-chondroitinase deletion mutant fusion proteins, Tat-GGF2 fusion proteins, or Tat-chondroitinase-GGF2 fusion proteins may be expressed from *E. coli*. Other fusion proteins can be expressed in CHO cell lines.

**[0044]** Table 1 illustrates various non-limiting components which may be used in compositions of the present invention as a mixture, and preferably as a fusion or chimeric molecule. The composition or chimeric molecule described may include one or more of the molecules in Table 1. In the case of chimeric molecules one or more linker segments, preferably polypeptides are used.

**Table 1 Components of Compositions**

Transduction molecules	Proteoglycan degrading molecules	Therapeutic, diagnostic, receptor antagonist molecules
Tat protein residues 48-57 HIV-1 Rev protein basic motif HIV-1 Rev protein basic motif Herpes simplex virus VP22 protein	Any agent that degrades extracellular matrix glycoproteins including:  Chondroitinase ABC I, Chondroitinase ABC II, Chondroitinase AC, Chondroitinase B, Hyaluronidase 1, Hyaluronidase 2, Hyaluronidase 3, Hyaluronidase 4, PH20  deletion and or substitution mutants of the above listed molecules that maintains enzymatic activity.	Any peptide that blocks the inhibitory properties of Nogo, MAG, OMgp including:  Nogo peptide 1 – 40* Any component of Nogo peptide 1-40 that maintains the ability to block the inhibitory properties of Nogo Other blocking peptides of Nogo Antibodies that recognize Nogo Blocking peptides of MAG Antibodies that recognize MAG Blocking peptides of OMgp Antibodies that recognize OMgp Antibodies that recognize the

		Nogo-66 receptor Blocking peptides of the p75 receptor Antibodies that recognize the p75 neurotrophin receptor Peptides or antibodies that block other receptors of Nogo, MAG and/or OMgp  Peptide that overcomes the inhibitory properties of Myelin, Nogo, MAG, OMgp including: Protein Kinase C family inhibitors* Rho Kinase family inhibitors Agents that increase intracellular cAMP concentration L1
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\*Nogo peptide 1-40: The human 40-aa NEP1-40 aa (Acetyl-RIY KGV IQA IQK SDE GHP FRA YLE SEV AIS EEL VQK YSN S-amide corresponding to 1-40 aa of Nogo-66

**[0045]** A schematic illustration of non-limiting versions of chimeric fusion proteins of the present invention are illustrated in FIG. 4A and FIG. 4B.

**[0046]** A peptide component from any column in Table 1 could be linked by an oligopeptide linker well known in the art such as a glycine rich peptide, for example Gly-Gly-Gly-Gly-Gly, or linkers prepared including an of the naturally occurring amino acids as well as substituted or beta or gamma amino acids like 4-aminobutyric acid or 6-aminocaproic acid can be used. Other linkers including but not limited to alkyl diamines, amino, or alkyl diols may also be used. Preferably the Transduction component of the fusion protein is in a terminal position in the chimeric protein. Other examples of common linkers may include but would not be limited to Gly-Gly-Ala-Gly-Gly, Gly/Ser rich linkers (for example Gly<sub>4</sub>Ser<sub>3</sub>), or Gly/Ala rich linkers.

Additionally, linkers may be of any length and design to promote or restrict the mobility of components in the fusion protein.

[0047] The incorporation of non-natural amino acids, including synthetic non-native amino acids, substituted amino acids, or one or more D-amino acids into the peptides (or other components of the complexes) of the present invention (subsequently referred to herein as "D-peptides") is advantageous in a number of different ways. D-amino acid-containing peptides exhibit increased stability *in vitro* or *in vivo* compared to L-amino acid-containing counterparts. Thus, the construction of peptides incorporating D-amino acids can be particularly useful when greater intracellular stability is desired or required. More specifically, D-peptides are resistant to endogenous peptidases and proteases, thereby providing better oral transepithelial and transdermal delivery of linked drugs and conjugates, improved bioavailability of membrane-permeant complexes, and prolonged intravascular and interstitial lifetimes when such properties are desirable. The use of D-peptides can also enhance transdermal and oral transepithelial delivery of linked drugs and other cargo molecules.

[0048] In a spinal cord injury, the axons of ascending sensory and descending motor neurons are disrupted, that can result in the loss of sensation and paralysis. These axons fail to regenerate successfully leading to permanent disability. A scar envelopes the site of the injury which is believed to wall off the area of fragile tissue, stabilize the blood brain barrier, and prevent an overwhelming cascade of uncontrolled tissue damage. This scar is composed of hypertrophic glial cells and an extracellular matrix (ECM). Chondroitin sulfate proteoglycans (CSPGs) are one important component of the scar. They are expressed by glial cells and deposited in the ECM in regions of blood brain barrier breakdown. *In vitro* evidence demonstrates that these CSPGs are potently inhibitory for the growth of axons and without



wishing to be bound by theory, are believed to contribute to the failure of the spinal cord axons to regenerate and reform functional synapses. *In vivo* studies have demonstrated that regenerating axons are able to grow into and even beyond the scar.

**[0049]** CSPGs and white matter components are generally accepted as molecular barriers that neurons must overcome in order to regenerate and reestablish functional connections after injury. Transplanted adult sensory neurons placed distal to a forming scar can regenerate robustly even along degenerating white matter pathways, however, regeneration ceases abruptly as axons enter the proteoglycan containing glial scar. Treatment of CNS white matter pathways with chondroitinase enhances the ability of neurons to grow in these substrates.

**[0050]** Central nervous system tissues are tightly compacted with cells and have limited extracellular space. The proteins and carbohydrates of the extracellular matrix provide charge and osmotic forces as well as specific and non-specific binding sites, which may prevent the penetration of therapeutic agents. The enzymatic cleavage of these matrix and cellular components may later or facilitate the access of compounds or cells through tissues. A proteoglycan degrading molecule like Chondroitinase ABC I that is an enzyme that digests chondroitin sulfate proteoglycans can be used to promote diffusion of therapeutic molecules into the CNS. Tat peptides to transport covalently linked biologically active cargo molecules into the cytoplasm and nuclei of cells. In the case of a fusion protein having a protein transduction polypeptide domain like the HIV tat protein, therapeutic molecules for axon regeneration may be delivered across the blood brain barrier.

**[0051]** Treatment of SCI model injuries, preferably contusion model injury, can be used to determine the degree of regeneration and functional recovery achieved by compositions and method of the present invention. The degree of functional recovery can be demonstrated by

improved corticospinal tract conduction, improved tape removal, beam walking, grid walking and paw placement following chondroitinase treatment of a dorsal column lesion. Motor skill improvement as well as autonomic function: bowel, bladder, sensor and sexual function may also be used as measures of function improvement and related to molecular structure and components in the compositions of the present invention.

**[0052]** In addition to the ability of chondroitinase to enhance regeneration, chondroitinase digests components of the perineuronal network (PNN). The density of the PNN is variable within the CNS and is particularly dense in the somatosensory, auditory, visual cortices and the hippocampus. PNN has also been demonstrated to be dense around spinal motor neurons. The inventors have discovered the dense PNN within the dorsal horn of the cord. Digestion of the PNN can enhance plasticity within the hippocampus and visual cortex. Plasticity within intact systems in incomplete SCI, especially in the region of the central pattern generators or in the reticular core, may support the function of damaged or destroyed systems. Promotion of plasticity in these systems may be one mechanism other than or in addition to regeneration by which chondroitinase can improve function following CNS injury. Furthermore, regeneration and plasticity may work in concert to affect recovery following injury; indeed, the corticospinal tract has been shown to be critical for modulation of spinal cord plasticity.

**[0053]** Recovery of neurological function following contusion injury in the CNS or a disease state may be promoted by administering the fusion proteins or mixtures including one or more of the components in Table 1, to cells, a tissue, or a subject having damaged or diseased neurons whether the injury or disease is immediate or long-standing.

**[0054]** The fusion proteins herein are administered in an amount effective to degrade CSPGs and thereby promote the recovery of neurological function. Once the proteins or

polypeptides in the compositions have been purified to the extent desired, they may be suspended or diluted in an appropriate physiological carrier or excipient for SCI treatment. In models of SCI, effective intrathecal doses in rats have been about 0.06 units on alternate days for 14 days. A dose for a 70 kilogram human may be about 17 Units. At about 100 Units / milligram, this would equal about 170 micrograms. Doses of up to 20 Units appear safe in the rat. Compositions including a proteoglycan degrading molecule in a mixture or as part of a fusion protein diluted in a carrier or pharmaceutically acceptable excipient can be injected, generally at concentrations in the range of 1  $\mu$ g to 500 mg/kg of host. Administering the agent can be by bolus injection, intravenous delivery, continuous infusion, sustained release from implants, or sustained release pharmaceuticals. Administration may be by injection, such as intramuscularly, peritoneally, subcutaneously, intravenously. Oral administration may include tablets or capsules, preferably the oral dosage is a sustained release formulation for once or twice daily administration. Percutaneous administration can be once per day, and is preferably less than once per day administration. Administration to the human patient or other mammalian subject may be continued until a measurable improvement in autonomic or motor function in the patient is achieved.

[0055] The chondroitinase PTD fusion proteins can be administered with a suitable pharmaceutical carrier. The administration of the compositions of the present invention as mixtures or chimeric proteins can be topical, local or systemic. The chimeric fusion proteins may also be secreted by genetically cells, preferably a chimeric fusion protein having a proteoglycan degrading portion like chondroitinase and a transduction polypeptide portion like TAT can be secreted by genetically modified cells that are implanted, either free or in a capsule, at or near the site of CNS injury.

**[0056]** Once the compositions either as mixtures or fusion proteins are administered, degradation of CSPGs removes the inhibitory molecules that block neurite outgrowth, and allow the regeneration of neurites into the affected area. For example, the chondroitinase AC and chondroitinase B degrade CS and DS, respectively, resulting in unsaturated sulfated disaccharides. Chondroitinase AC cleaves CS at 1, 4 glycosidic linkages between N-acetylgalactosamine and glucuronic acid in the polysaccharide backbone of CS. Cleavage occurs through beta-elimination in a random endolytic action pattern. Chondroitinase B cleaves the 1, 4 galactosamine iduronic acid linkage in the polysaccharide backbone of DS. The cleavage of both CS and DS occurs through a beta-elimination process which differentiates these enzymatic mechanisms from mammalian GAG degrading enzymes. Chondroitinase ABCI, chondroitinase ABCII, are exo and endo lyases that cleave both CS and DS. The removal of CS and DS from the glial scar permits the regeneration of neurite outgrowths into the injured area.

**[0057]** Mixtures of any of these fusion polypeptides may be used to provide a therapeutic treatment for CNS injuries and disorders which may include but not limited to contusion injury, traumatic brain injury, stroke, multiple sclerosis, brachial plexus injury, ambliopia, spinal cord injuries. Spinal cord injuries includes disease and traumatic injuries, such as the crushing of neurons brought about by an auto accident, fall, contusion, or bullet wound, as well as other injuries. Practice of the present methods can confer clinical benefits to the treated mammal, providing clinically relevant improvements in at least one of the subject's motor coordination functions and sensory perception. Clinically relevant improvements can range from a detectable improvement to a complete restoration of an impaired or lost function of the CNS.

**[0058]** The regeneration of the nerve cells in to the affected CNS area allows the return of motor and sensory function. Clinically relevant improvement will range from a detectable

improvement to a complete restoration of an impaired or lost nervous function, varying with the individual patients and injuries.

**[0059]** A variant of a protein or fragments thereof refer to a molecule substantially similar to either the entire protein or a fragment, which possesses biological activity that is substantially similar to a biological activity of the complement protein or fragments. A molecule is substantially similar to another molecule if both molecules have substantially similar structures or if both molecules possess a similar biological activity.

**[0060]** Variants of complement protein or fragments thereof are produced by chemical or recombinant means. Variants of the polynucleotides constructed to express fusion proteins may also be made. The variants may include, for example, deletions from, or insertions or substitutions of, amino acid residues within the amino acid sequence, or deletion, substitution, or insertion of nucleic acids from a sequence encoding for a particular fusion protein or polypeptide domain in the fusion protein. For example, in some cases the removal of one or more amino acid residues from a chondroitinase polypeptide can be made without significant change in its CSPG degradation activity. Substantial changes in functional properties like proteoglycan degradation or blocking activity against axon growth inhibitors are made by selecting substitutions that are less conservative, ie. that differ more significantly in their effect on maintaining the structure, charge or hydrophobicity of the peptide backbone in the area of the substitution.

**[0061]** Most deletions, insertions, and substitutions are not expected to produce radical changes in the characteristics of the protein molecule; however, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. For example, a

change in the axon regeneration character of the polypeptide molecule, through more or less proteoglycan degradation can be measured with functional recovery tests as well as HPLC assays to detect CSPG disaccharide digestion products.

[0062] The present invention relates to the use of a proteoglycan degrading molecule, an HIV tat protein, or a tat-derived polypeptide, or a combination of these as a mixture or fusion protein to deliver a molecule of interest into the CNS or a site in the CNS where neuronal tissue damage has occurred by disease or trauma. In particular the damage site in the CNS is where scarring has occurred as a result of a contusion injury. The molecule of interest, optionally referred to as an agent or cargo molecule can be a therapeutic molecule which promotes plasticity, axon growth, a diagnostic molecule, or a proteoglycan degrading molecule. In the case of a fusion protein having a protein transduction polypeptide domain like the HIV tat protein, therapeutic molecules for axon regeneration may be delivered across the blood brain barrier or proteoglycan degrading molecule can be delivered into cells to degrade cellular stores of proteoglycans and promote axon regeneration.

[0063] Transport polypeptides of this invention may be advantageously attached to cargo molecules by chemical cross-linking or by genetic fusion. A unique terminal cysteine residue is a preferred means of chemical cross-linking. According to some preferred embodiments of this invention, the carboxy terminus of the transport moiety is genetically fused to the amino terminus of the cargo moiety. Embodiment of the present invention consists of an amino-terminal methionine followed by tat residues 47-58, followed by a chondroitinase polypeptide.

[0064] It will be appreciated that the entire 86 amino acids which make up the tat protein may not be required for the uptake activity of tat. For example, a protein fragment or a peptide which has fewer than the 86 amino acids, **but which exhibits uptake into cells and or can**

**cross the blood brain barrier**, can be used (a functionally effective fragment or portion of tat). For example, tat protein containing residues 1-72 can be sufficient for uptake activity and tat residues 1-67 can mediate the entry of a heterologous protein into cells. Synthetic peptide containing tat residues 1-58 can have uptake activity.

[0065] The tat peptide can be a single (i.e., continuous) amino acid sequence present in tat protein or it can be two or more amino acid sequences which are present in tat protein, but in the naturally-occurring protein are separated by other amino acid sequences. As used herein, tat protein includes a naturally-occurring amino acid sequence which is the same as that of naturally-occurring tat protein, its functional equivalent or functionally equivalent fragments thereof (peptides). Such functional equivalents or functionally equivalent fragments can possess uptake activity into the cell or across the blood brain barrier that is substantially similar to that of naturally-occurring tat protein. Tat protein can be obtained from naturally-occurring sources or can be produced using genetic engineering techniques or chemical synthesis.

[0066] The amino acid sequence of naturally-occurring HIV tat protein can be modified, by addition, deletion and/or substitution of at least one amino acid present in the naturally-occurring tat protein, to produce modified tat protein (also referred to herein as tat protein or polypeptide). Modified tat protein or tat peptide analogs with increased stability can thus be produced using known techniques. Therefore, tat proteins or peptides may have amino acid sequences which are substantially similar, although not identical, to that of naturally-occurring tat protein or portions thereof. In addition, cholesterol or other lipid derivatives can be added to tat protein to produce a modified tat having increased membrane solubility.

[0067] Naturally-occurring HIV-1 tat protein has a region (amino acids 22-37) wherein 7 out of 16 amino acids are cysteine. Those cysteine residues are capable of forming disulfide

bonds with each other, with cysteine residues in the cysteine-rich region of other tat protein molecules and with cysteine residues in a cargo protein or the cargo moiety of a conjugate. Such disulfide bond formation can cause loss of the cargo's biological activity. Furthermore, even if there is no potential for disulfide bonding to the cargo moiety (for example, when the cargo protein has no cysteine residues), disulfide bond formation between transport polypeptides can lead to aggregation and insolubility of the transport polypeptide, the transport polypeptide-cargo conjugate, or both. The tat cysteine-rich region may be deleted to avoid disulfide bond formation and prevent aggregation and insolubility of the transport polypeptide, the transport polypeptide-cargo conjugate, or both.

[0068] Chondroitinase is also able to promote plasticity in regions of the CNS with significantly dense PNN, including cortex, tectum, hippocampus and spinal cord. It is reasonable that some combination of effects, including regeneration, sprouting and plasticity are responsible for the improvement in function following SCI with chondroitinase treatment or treatment with fusion molecules including chondroitinase or other proteoglycan degrading molecule.

[0069] NbR<sub>27-311</sub>: Nogo is a high molecular weight myelin component that inhibits neurite outgrowth. The amino terminal region (Nogo66) is the part of the molecule that is specifically associated with the inhibition of neurite outgrowth. Expression cloning methods revealed the receptor for Nogo66 (NgR) is a GPI anchored glycoprotein expressed mainly on neurons. NgR interacts not only with Nogo, but also with other myelin associated inhibitors such as MAG and MOG. Due to its central role in the inhibitory properties of myelin on neurons, NgR has been the target for approaches to antagonize its interactions with its ligands. The soluble segment of NgR interacts with Nogo66, MAG and MOG. The region spans residues



27-311 and this NgR fragment can therefore act as a decoy receptor that will interfere with myelin associated inhibition of neurons. If NgR<sub>27-311</sub> is linked to proteoglycan degrading molecule like chondroitinase ABCI to form a chimeric fusion protein, the NgR<sub>27-311</sub> polypeptide domain of the fusion protein would be expected to limit the myelin-associated inhibition of neurite outgrowth and promote axonal regeneration in chondroitinase digested regions of a spinal cord injury.

[0070] For cloned (NgR<sub>27-311</sub>), the precise region of interest, or fragments can be derived from the initial clone. The biological activity of NgR<sub>27-311</sub>, its fragments, and fusion polypeptides including NgR<sub>27-311</sub> may be confirmed using an in vitro assay for the collapse of growth cones on neurons. The growth cone collapse assay has been established and the addition of MAG or Nogo66 causes the collapse of growth cones in a dose dependent manner. If NgR<sub>27-311</sub>, its fragments, and fusion polypeptides including NgR<sub>27-311</sub> are active is biologically active then they should inhibit the MAG and Nogo66 mediated growth cone collapse. Growth cone collapse data may be collected by the inspection of photomicrographs of DRG neurons in response to Nogo66 and MAG.

[0071] The L1 polypeptide is a member of the immunoglobulin superfamily of cell adhesion molecules and is expressed in growing axons, glial progenitor cells and Schwann cells throughout life, but has only limited expression in the CNS. L1 interacts with itself and with other extracellular molecules, such as the FGF receptor to promote neurite fasciculation and growth. Expression of L1 is generally associated with a permissive environment for axonal regeneration the, precise region of interest, or fragments of L1 can be derived from the initial clone. For example, Schwann cells that express L1 support peripheral nerve regeneration and axonal growth is observed in the optic nerves from transgenic mice that express L1 in astrocytes

but not in wild-type optic nerves. Fibroblasts engineered to express L1 support axonal growth when transplanted into spinal cord. Finally, a soluble form of L1 linked to Fc promoted functional recovery following acute SCI. If L1 is linked to proteoglycan degrading molecule like chondroitinase ABCI in a fusion protein it is reasonable to expect the fusion protein to promote axonal regeneration in chondroitinase digested regions of a spinal chord injury.

[0072] The neuregulins and their receptors comprise a diverse growth factor and receptor tyrosine kinase system that has been demonstrated to be essential for organogenesis in the CNS, muscle epithelial and other tissues. GGF2 is a soluble isoform of the neuregulin 1 gene. It was initially characterized as a Schwann cell mitogen 32, but subsequent studies have demonstrated direct actions on oligodendrocytes, neurons and other cell types. GGF2 diminishes demyelination and inflammation and enhances remyelination in a mouse model for multiple sclerosis. Based on these results, it is reasonable to expect that a recombinant human GGF2 is a potential treatment for demyelination associated with SCI. With GGF2 is linked to a proteoglycan degrading molecule like chondroitinase ABCI it is reasonable to expect to promote remyelination of axons in chondroitinase-digested regions of a SCI.

Table 2. Examples of non-limiting GGF2 fragments for expression in *E. coli* for use in compositions and fusion polypeptide compositions.

Construct	Domain	Nucleotide Seq	Amino Acid Seq	Mol. Wt.
GGF2-FL	Full length GGF2		M1-E422	46kDa
GGF2/1	Ig domain +EGF domain	748-1206	L250-C402	16.8 kDa
GGF2/2	Ig domain to C-terminus	748-1266	L250-E422	19 kDa

GGF2/3	EGF domain	1048-1206	T350-C402	5.7 kDa
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[0073] The efficacy of a composition of the present invention, such as a proteoglycan degrading molecule and a molecule that blocks the activity of an axon growth inhibitor either as a mixture of as a fusion protein can be evaluated using a validated rat SCI model at three different levels of SCI severity.

[0074] A proteoglycan degrading molecule like Chondroitinase ABCI is commercially available in small quantities as a naturally derived enzyme (Seikagaku Corporation) and can be made by a recombinant production system to have essentially the same activity as the enzyme purified from *Proteus vulgaris*. Genomic DNA can be isolated from *Proteus vulgaris* using DNeasy Tissue kit (Qiagen). PCR primers can be synthesized with an NdeI restriction site at the 5' end and a BamHI site at the 3' end having sequences 5'- CAT ATG GCC ACC AGC MT CCT GCA TTT G-3'(F2) and 5'- GGA TCC TCA AGG GAG TGG CGA GAG-3'(R) respectively, to synthesize the mature protein. The 3.0 kb PCR products can be ligated into pCR 2.1 vector (TOPO cloning kit, Invitrogen) and transformed into DH5a competent cells (Invitrogen). Plasmid DNA can be isolated from a number of clones screened by digestion with EcoRI restriction enzyme. The integrity of a gene prepared in this way can be confirmed by repeated DNA sequencing.

[0075] The chondroitinase ABCI sequence can be cloned into a PET vector (Novogen) for expression in *E. Coli*. After induction of gene expression with IPTG the bacteria can be lysed by sonication with the concomitant extraction of chondroitinase ABCI with Triton X-114/PBS. The inventors discovered that the majority of recombinant chondroitinase ABCI was found in the cytosolic fraction of the bacterial cell lysate that enabled the development of a chondroitinase ABCI purification protocol that yields an enzyme with high activity at high yields. The protocol

includes cation-exchange chromatography as a capture step and gel filtration as a polishing step. After these steps chondroitinase ABCI reaches a purity of ~95%. Anion exchange membrane filtration (Intercept Q, Millipore) can be used for endotoxin and host DNA removal. This step is expected to remove approximately 75% of the endotoxin. Following filtration, chondroitinase ABCI can be dialyzed into volatile buffer, pH 8.0 and lyophilized to dryness. The final product is stable at -70°C for long term storage. The purified cABCI is a highly basic protein with pI~9.5 as determined by IEF-PAGE analysis of the samples from the crude cell lysate.

[0076] A variety of analytical methods can be used to compare the enzymatic activity of the recombinant version of chondroitinase ABCI to that of a commercially available form of the enzyme (Seikagaku Corporation) purified from *Proteus vulgaris*. The methods may be adapted to evaluate the activity of fusion proteins including proteoglycan degrading polypeptides like chondroitinase. Specific activity measurements were obtained using an accepted spectrophotometric assay that measures the change in absorbance due to the production of reaction products from the degradation of proteoglycans. The recombinant form of chondroitinase ABCI had approximately 25% higher specific activity than the Seikagaku chondroitinase ABCI. Size exclusion chromatography can be used to compare the hydrodynamic properties the enzymes. The elution profiles for recombinant enzyme was identical to that of the naturally derived enzyme.

[0077] A form of zymography can used to further characterize the enzyme and may be adapted for characterization of the fusion proteins. Polyacrylamide gels can be polymerized in the presence of aggrecan, a substrate for chondroitinase ABCI. Enzyme samples may be resolved on the aggrecan-impregnated gels by electrophoresis in the presence of SDS. The gels can then be subjected to a renaturation step wherein the SDS was extracted and the enzymes

allowed to refold. Enzyme refolds and regains activity then digests aggrecan within the gel and the resulting loss of carbohydrate in that region of the gel can be visualized by a carbohydrate-specific stain. A similar loss of carbohydrate in the gel would be expected for active forms of a fusion protein including a proteoglycan degrading polypeptide portion. In the case of recombinant Chondroitinase ABCI, its activity can be visualized as a clear spot in the zymogram. The zymography results were consistent with the spectrophotometric analysis that demonstrates that the recombinant form of chondroitinase ABCI has the same or greater specific activity as the naturally occurring form.

**[0078]** HPLC methods may be used for detecting the four and six sulphated disaccharides ( $\Delta 4$ DS and  $\Delta 6$ DS, respectively) liberated as a result of chondroitinase ABCI digestion of CSPG. The two disaccharides can be effectively resolved by anion exchange chromatography. The HPLC assay has been validated by showing that the quantitation of  $\Delta 4$ DS and  $\Delta 6$ DS from chromatograms yields a linear relationship to the amounts injected into the HPLC. Production of  $\Delta 4$ DS and  $\Delta 6$ DS from CSPG digestion is directly related to the amount of chondroitinase specific activity as determined by the spectrophotometric assay described above. This assay may be used as a sensitive and accurate means to independently quantitate  $\Delta 4$ DS and  $\Delta 6$ DS released by chondroitinase digestion of a variety of substrates and may also be used to determine the activity of chondroitinase polypeptides in a fusion protein.

**[0079]** Another functional assay that can be performed to characterize proteoglycan polypeptide activity is where dorsal root ganglionic (DRG) neurons are plated on aggrecan or aggrecan treated with a proteoglycan like chondroitinase ABCI. It is expected that neurons plated on aggrecan will fail to adhere to the plate and extend axons. In contrast, neurons plated on aggrecan treated with a proteoglycan degrading polypeptide like chondroitinase ABCI

in a composition or as part of a fusion polypeptide would be expected to adhere to the surface and extend axons. The extensive axon growth, which is observed for chondroitinase ABCI is believed to be due to the digestion of the carbohydrates on the aggrecan core protein which creates a more permissive substrate for axon growth.

[0080] The rat contusion model of SCI is a clinically relevant model and may be used to evaluate the efficacy of fusion proteins and other compositions of the present invention for promoting axon regeneration. With a contusion SCI, cells are destroyed, hemorrhage ensues and inflammation begins. Destroyed cells are removed by macrophages and a reactive gliosis begins. A cystic cavity is formed and the gliosis matures into a glial scar. Myelin in the area is destroyed and many local neurons that are not destroyed are left in a demyelinated state.

[0081] The forceps compression model of SCI is a contusion model developed and characterized. This model has been validated and results in injuries that are very similar to the more widely used impactor models. The model can involve a forceps compression at vertebral level T9/T10. The forceps compress the cord to a width of 0.9, 1.3 or 1.7 mm for 15 seconds. These three levels of compression allow a severe, mild or moderate injury. This model has been validated using the open field locomotor testing and the Basso, Bresnahan and Beattie (BBB) scoring system. It was also characterized histologically. Behavioral testing and BBB scoring demonstrated that the forceps produce a highly reproducible injury, with recovery similar to that seen with impactor models. These testing and scoring data demonstrate that the forceps compression model is comparable to other contusion models and sufficiently reproducible to be used as an experimental model of SCI and may be used for the evaluation of compositions of the present invention..

**[0082]** Tissue from forceps compression injured animals can be processed histologically to examine white matter sparing, glial scar and cyst formation. As with other contusion SCI models, a central cyst is formed after injury, with a size that increases with increased injury severity (decrease forceps gap). Around the cyst a glial scar forms that is characterized by astrogliosis (GFAP), macrophage activation and myelin wasting.

**[0083]** Various aspects of the present invention will be illustrated with reference to the following non-limiting examples.

### **EXAMPLE 1**

**[0084]** This example illustrates how a proteoglycan degrading molecule may be administered, studied, and demonstrated to show a functional improvement in animals having a model contusion injury.

**[0085]** The forceps compression model of SCI is a contusion model developed and characterized. This model has been validated and results in injuries that are very similar to the more widely used impactor models. The model can involve a forceps compression at vertebral level T9/T10. The forceps compress the cord to a width of 0.9, 1.3 or 1.7 mm for 15 seconds. These three levels of compression allow a severe, mild or moderate injury.

**[0086]** Rats were injured with the forceps compression model at vertebral T9/T10/ At the time of surgery an intrathecal catheter was placed for delivery of chondroitinase. Animals were treated every day for one week and then on alternating days for one week with 0.06 U/ dose chondroitinase ABC I (Seikagaku), penicillinase or artificial cerebrospinal fluid (aCSF). Doses and controls were derived from Bradbury et al., 2002. Behavior was assessed using open-field locomotor testing and the BBB scoring system at day 2 and then weekly post injury for ten weeks.

[0087] FIG. 5A shown are mean BBB scores for animals treated with chondroitinase ABC I, penicillinase and aCSF. Rats treated with aCSF or penicillinase recovered to a mean BBB score of about 4. Rats treated with chondroitinase recovered to a mean BBB score of about 8. Multiple animals recovered to scores above 10, indicating supra-spinal input. The chondroitinase scores were significantly different from both control groups by ANOVA and post hoc Tukey.

[0088] Tissue from these animals was processed immunohistochemically for glial fibrillary acidic protein (GFAP) to assess general scar architecture. Tissue was also stained with a Weil stain and a silver degeneration stain to assess myelin and neuron degeneration, respectively. Interestingly no obvious differences in these parameters were noted between experimental and control treated tissues.

[0089] In FIG. 5B the large lesion comprising several segments and the sparing in the ventral cord. Weil staining revealed extensive demyelination in both the treated and untreated animals. The GFAP images (middle set) demonstrate the extent of the scar that is formed following forceps injury. The bottom amino cupric silver degeneration stain demonstrates the vast neural degeneration extending both rostrally and caudally after injury. Again, no obvious differences were noted between chondroitinase treated and control tissues.

[0090] Additional preliminary experiments have been performed at moderate injury levels and significant improvement in open-field locomotor activity was observed with chondroitinase treatment. Animals receiving chondroitinase ABCI recovered to a mean BBB score of 9.1 at ten weeks following injury, compared with 7.1 for the penicillinase controls. The consistency of the data (SEM's of 0.6 and 0.3, respectively) and the region of scores on the BBB scale make this 2 point change not only statistically significant, but also clinically meaningful. A



score of 9 indicates plantar placement with weight support or frequent to consistent weight support with dorsal stepping while a score of 7 indicates movement of each joint in hind limb but with no weight support and no consistent sweeping of limbs. An examination of the individual animal scores at ten weeks shows that 6 of 12 animals in the chondroitinase group recovered to scores of 9 or above, while only one of 12 animals in the penicillinase group recovered to a score of 9. One animal from each group was removed from analysis because its score failed to ever rise above 2.5, indicating an injury severity outside the model norms. FIG. 5C and FIG. 5D includes a scatter plot of scores at 10 weeks for each animal in the penicillinase and chondroitinase treatment groups for the moderate injury. Group means are shown below.

[0091] The results show in this well controlled study, that chondroitinase improves open-field locomotor function in Rats that were injured with the forceps compression model at vertebral T9/T10. Animals recovered to mean BBB scores of 9.7 and 9.9 for the penicillinase and chondroitinase groups, respectively. This study demonstrates a significant effect at severe and moderate injury levels, but not mild injury levels. No significant differences were noted between any groups in the mild injury study (1.3 mm forceps). It is unclear if chondroitinase is not effective with mild injury, if chondroitinase effected changes not adequately assayed by the open-field locomotor testing such as stride length, paw placement, sensory or autonomic functions. Preliminary analysis of histology from animals in this study confirmed placement of the catheters and injuries in each animal. Ongoing experiments sacrificing animals at time points after injury with and without chondroitinase treatment will characterize the CSPG content of the scar and the effects of chondroitinase digestion on stub-antigen expression. Future experiments will expand the battery of behavior tests and examine the cord for evidence of regeneration with tract tracing.

[0092] Two acute toxicity studies were conducted in rats. The first was an intravenous (IV) study wherein rats were injected with 0, 0.2, 0.775 or 7.775 mg/kg chondroitinase ABCI. In the second study, intrathecal (IT) catheters were placed over the spinal cords using the same methods employed in the SCI animal studies and 0.06, 0.6 and 6.0 units of chondroitinase ABCI was delivered through the IT catheters. These doses were 1, 10, and 100-fold greater than the estimated local concentrations of chondroitinase ABCI achieved with IT catheters in the SCI experiments. Animals were monitored for pain and distress and body weights were acquired daily. No overt reactions were observed during or immediately after chondroitinase ABCI dosing. No swelling, inflammation, bruising or necrosis was noted at the injection site for the IV experiment. No changes in body temperature were observed in animals treated via the IT route. No alterations in feeding, grooming or vocalizations were noted. Animals were assessed for motor behavior in an open pool. No abnormalities were noted by the animal care staff or behavioral specialists. Animals displayed no signs of joint tenderness or swelling. There were no significant differences in weight change between the treatment groups. The results demonstrate that chondroitinase ABCI treatment is not associated with acute toxicity using IV and IT doses substantially greater than the efficacious IT doses.

## EXAMPLE 2

[0093] This example describes the preparation of Nogo-receptor agonist, L1 neural cell adhesion protein, and GGF2 polypeptide domains which can be used for compositions and fusion proteins of the present invention.

[0094] A soluble portion of the human Nogo receptor spanning amino acids 27 to 311 (NgR<sub>27-311</sub>) was selected as it has been shown to inhibit the binding of Nogo66, MAG, and MOG to membrane-bound NgR. Primers were designed flanking this region, and RT-PCR was

performed using human hippocampal RNA (BD Biosciences). The 1.05kb region was successfully amplified and purified.

[0095] L1 was prepared through a CHO cell line from the laboratory of Dr. Melitta Schachner that secretes human L1 as a fusion protein with human Fc (L1-Fc). The cells were grown in roller bottles and then L1-Fc was purified from the conditioned media using protein A affinity column chromatography. The purity of L1-Fc was assessed by SDS-PAGE and a single band at the appropriate molecular weight was observed. The biological activity of L1 -Fc was confirmed using a neurite outgrowth assay. Tissue culture plates were coated with either poly L-Lysine or L1-Fc and then cerebellar granule cells from postnatal day 10 rats were isolated and placed into culture on the substrates. It was observed that neurons plated on the L1-Fc substrate exhibited a substantial number of long neuritis compared to the polylysine substrate controls. These results demonstrate that the L1-Fc produced is biologically active in promoting neurite outgrowth. This neurite outgrowth assay will be used to assess the biological activity related to the L1 portion of the chondroitinase ABCI fusion protein..

[0096] A CHO cell line that secretes a soluble form of full length GGF2 glycoprotein was obtained from CeNes. Extensive optimization of media and purification methods was completed to obtain essentially pure and biologically active GGF2. A number of analytical methods were developed to characterize the GGF2 including SDS-PAGE, isoelectric focusing, peptide mapping and carbohydrate analysis. For example an SDS-PAGE gel of reduced and non-reduced GGF2; the isolate is essentially free of contaminating proteins and shows the expected molecular weight and monomeric structure. The biological activity of GGF2 was assayed using a primary rat Schwann cell proliferation method and the expected effect was reproducibly obtained with four independent batches of GGF2. Another functional assay was

developed that measures the phosphorylation of Akt kinase, a downstream cell signaling component of the erbB receptor pathway. It was observed that there is a dose dependent phosphorylation of Akt kinase

### EXAMPLE 3

[0097] This example illustrates the manipulation of chondroitinase ABCI for construction of chimeras.

[0098] Chondroitinase ABCI was cloned from *P.vulgans* and expressed in *E.coli*. Surprisingly repeated attempts to create N-terminal fusion proteins were not successful because the N-Terminal fusion portion was cleaved from chondroitinase ABCI during synthesis. However, catalytically active deletion mutants with N-terminal His fusion tags designated ABC I-NΔ20, ABC I-NΔ40 and ABCI-NΔ60 were prepared by deleting 20, 40 and 60 amino acids respectively from the N-terminus of the mature ABCI protein. Unlike the full length chondroitinase ABCI, the N-terminal deletion mutants are capable of synthesizing a 6xHis tag as an N-terminal fusion protein. It was also observed that deletion of 80 amino acids from the C-terminal end formed a mutant with proteoglycan degrading activity of chondroitinase ABCI as tested in a zymography assay.

[0099] Fusion proteins with NgR<sub>27-311</sub> or L1 with a proteoglycan degrading molecule such as chondroitinase ABCI will utilize mammalian expression and can be performed in CHO cells. The cDNA for chondroitinase ABCI has been cloned in pSECTag vector (Invitrogen) in the proper reading frame. A CHO cell line having secretory chondroitinase ABCI can be developed and the conditioned medium can be tested for catalytic activity by zymography assay to confirm that chondroitinase ABCI expressed in mammalian cells is functional. A mammalian

cell codon optimized version of chondroitinase ABCI can be synthesized by methods known in the art for use for CHO cell expression.

**[00100]** GGF2 is a spliced variant of the NRG1 gene expressed in brain and spinal cord of adult humans. It is a glycosylated protein of molecular mass between 66-90 kDa. The inventors have discovered that recombinant GGF2 expressed in CHO cells is highly glycosylated and promotes Schwann cell proliferation in vitro and further that an EGF-like domain of NRG1 expressed in *E.coli* is fully functional in promoting myocyte proliferation and survival.

**[00101]** The inventors have expressed fragments of GGF2 in *E.coli* as described in the preliminary data section. The specific GGF2 domain responsible for Schwann cell proliferation and thereby remyelination can be determined. If the Ig and EGF domains together or separately show biological activity in vitro, then they can be used to form chimeric fusion proteins.

**[00102]** Cloning and expression of NgR<sub>27-311</sub> in CHO cells. An NgR fragment corresponding to residues 1-359 was isolated by RT-PCR from human hippocampus poly K RNA (BD Biosciences) and its structure can be confirmed by DNA sequencing. The gene fragment corresponding to residues 27-311 can be cloned from the larger fragment and then subcloned in pSECTag vector (Invitrogen) in the proper reading frame to express the fragment as a secretory protein in CHO cells. The plasmid DNA containing the NgR<sub>27-311</sub> gene can be transfected in CHO cells and the cell line producing NgR<sub>27-311</sub> can be selected under the selection pressure of hygromycin B. An NgR<sub>27-311</sub>-ABCI chimera expression plasmid can be constructed for expression in a CHO cell expression system using methods known in the art.

#### EXAMPLE 4

[00103] This example illustrates methods which may be used to purify and isolate expressed chondroitinase ABCI, and GGF2 domains expressed in *E. coli*. These method may be applied to purification of chimeric fusion proteins of the present invention.

[00104] An efficient *E. coli* recombinant expression system and a purification process for chondroitinase ABCI have been developed by the inventors that could be applied for the purification of chondroitinase ABCI chimeric derivatives.

[00105] For example, the expression of various GGF2 domains in the chondroitinase ABCI expression host, *E. Coli*, has been preformed and the following peptides have been tested: aa250-402, aa250-422 and aa350-402. These expressed peptides were found in the soluble fraction of the *E. coli* lysates. It is reasonable to expect that final chimeric products of these peptides with chondroitinase ABCI in *E. coli* will also be soluble. In addition, it is expected that the charge characteristics of the chimeric products when compared to the recombinant chondroitinase ABCI will be similar. Thus, the theoretical isoelectric point (pI) values for the GGF2 peptides are 9.3 for aa250-402, 9.18 for aa250-422 and 7.55 for aa350-402. Fusing the first two peptides with chondroitinase ABCI is expected to result in chimeric proteins with pI values still above 9. In this case SP chromatography is expected to perform well as the capturing step. The smallest GGF2 peptide, aa 350-402, will reduce the pI of the final chondroitinase ABCI chimera to approximately 8.4. This change may require optimization of the capture step conditions.

[00106] The chimeric products of chondroitinase ABCI with NgR<sub>27-311</sub> and L1 peptides can be expressed in a CHO cell system. Prior to purification of the expressed chimeric proteins, the growth conditions for the cell line producing either the NgR<sub>27-311</sub> or L1 chimeric proteins will be optimized in serum-free medium. A detailed media optimization study can be

performed to determine the highest production conditions. Scale-up volume can be decided based on the rate of production of the chimeric proteins (pg/cell/day). Conditioned media from the various chimera-producing cell lines can be collected and subjected to tangential flow filtration. Ion-exchange chromatography can be used for capturing the secreted proteins from conditioned media, gel filtration chromatography can be used as a polishing purification step and then anion-exchange membrane filtration can be used for endotoxin and DNA removal. At each step the efficiency of the purification will be analyzed by SDS-PAGE, and spectrophotometric quantitation of protein concentration.

### EXAMPLE 5

**[00107]** This prophetic example describes in vitro assessment of chimera biological activity: Each chimera can be assayed for chondroitinase enzymatic activity and the specific biological activity of each fusion partner.

**[00108]** The first step in analysis may employ conventional protein biochemical methodologies to confirm the fidelity of gene expression. These include SDS-PAGE, IEE, mass spectrometry and size exclusion chromatography.

**[00109]** Chondroitinase chimera specific activity can be determined using a standard and uniformly accepted spectrophotometric assay. The production of reaction products from the catalytic activity of a chondroitinase chimeric polypeptide can be determined by a measurement of the absorbance of the product at a wavelength of 232 nm. A typical reaction mixture consists of 120 microliters of reaction mixture (40mM Tris, pH 8.0, 40mM NaAcetate, 0.002% casein) combined with substrate (5 microliters of 50 mM chondroitin C) and 1.5 microliters of chondroitinase ABCI chimeric fusion polypeptide or test sample. The change in absorbance units is the initial rate which can be converted to a unit activity measurement.

**[00110]** Disaccharide HPLC assay: The catalytic activity of chondroitinase chimeric polypeptide on a CSPG substrate is expected to release two species of sulphated disaccharides including  $\alpha$ - $\Delta$  AUA-[1 $\rightarrow$ 3]-GalNAc-4S ( $\Delta$  4DS) and  $\alpha$ - $\Delta$  AUA-[1 $\rightarrow$ 3]-GalNAc-6S ( $\Delta$  6DS). These species can be resolved by HPLC and the quantitation from the resulting chromatograms is a sensitive and accurate measure of chondroitinase activity. To perform the assay, samples from chondroitinase digestion reactions carried out with wild-type chondroitinase and chondroitinase fusion proteins can be clarified by centrifugation and then subjected to a validated anion exchange HPLC method as follows. A Dionex CarboPac PA-10 analytical column (4 x 250mm) fitted with a Dionex CarboPac PA-10 (4x 50mm) guard column can be used with a mobile phase consisting of a gradient of water at pH 3.5 (Buffer A) and 2M NaCl, at pH 3.5 (Buffer B). Detection may be set at a wavelength of 232 nm. A flow rate of 1 mL/minute and a 45 minute continuous gradient of 100% A to 100% B affords acceptable resolution of  $\Delta$ 4DS and  $\Delta$  6DS. Standard curves can be generated using known amounts of  $\Delta$ 4DS and  $\Delta$ 6DS.

**[00111]** Zymography allows resolution of proteins by molecular weight with a concomitant assessment of chondroitinase activity. A 10% polyacrylamide gel may be polymerized in the presence of 85pg/ml of aggrecan. Samples can be boiled in an SDS-loading buffer and then the analytes can be resolved by electrophoresis. After separation the gel can be incubated for 1 hour at room temperature in 2.5% Triton X100 then 16 hours at 37°C in fresh 2.5% Triton X-100. During these incubations, the SOS can be extracted from the gel and the chondroitinase refolds and digests the aggrecan in its immediate vicinity. After the refolding process the gel can be stained for carbohydrate: The gel can be first incubated in 0.2% Cetylpyridinium for 90 minutes at room temperature and then transferred into 0.2% Toluidine



Blue in 49:50:1 H<sub>2</sub>O, Ethanol, and Acetic Acid for 30 minutes. The gel can then be fully destained. Following destaining the gel can be incubated overnight in a 50 microgram/ml solution of Stains-All (Sigma) in 50% ethanol. Chondroitinase activity can be detected as a clear spot in the gel that is coincident with the molecular weight of the enzyme. The size of the clearing has been shown to be almost linearly related to Unit activity.

**[00112]** The NgR<sub>27-311</sub>-chondroitinase chimera can be assessed for the activity of the decoy Nogo receptor. The assay can be used to measure the collapse of growth cones: Dorsal root ganglia (DRG) are dissected from postnatal day 1 (P1) Sprague Dawley rat pups and dissociated in 200 U/ml collagenase I (Worthington) and 2.5 U/ml dispase (Boehringer/Roche) 2 times for 30 min. at 37°C. Enzymes are removed and DNase (0.5mg/ml) can be added to the ganglia. Trituration may be done with a pipette tip attached to a 1000 µl Pipetman. The resulting cell suspension can be filtered through a 40 micron cell filter and centrifuged at 70xg for 5 min. Cells can be re-suspended in DMEM/10% FBS and pre-plated for 2 hours on a non-coated tissue culture plate (100mm diameter). Non-adherent neurons are removed and plated at 10,000 cells/well in a Poly-lysine/laminin-coated 24 well plate in serum-free Neurobasal/B27 with 50ng/ml NGF. After 20 to 24 hours, MAG or Nogo66 can be added at varying concentrations for 1 hour at 37°C to induce growth cone collapse. NgR<sub>27-311</sub>-chondroitinase chimera can be added at various concentrations to compete with MAG and Nogo66 and thus protect the neurons from growth cone collapse. Cultures may be fixed by adding an equal volume of pre-warmed 8% paraformaldehyde/0.6 M sucrose to the medium for 20 min. while the cells are kept on a 37°C hot plate. Growth cones may be labeled with AlexaS68 phalloidin (Molecular Probes). Briefly, cells can be permeabilized with 0.1% Triton-X 100 for 5 min. at RT, blocked for 20min. in 1% BSA in PBS and incubated in phalloidin, diluted 1:40 in 1% BSA,

in PBS for 20min at RT. Cells can be washed in PBS and mounted in Fluorescent Mounting Medium (DAKO). The percentage of collapsed growth cones is determined by analyzing a minimum of 100 growth cones per well under a 40x objective.

**[00113]** The biological activity L1 fusion proteins may be determined using a standardized neurite outgrowth assay. After etching a 25mm circle in the center of a tissue culture-treated 35mm dish, 1 ml of 10 µg/ml poly-lysine can be added to each etched circle and incubated at 37°C for 60 minutes. The poly-lysine provides a negative control for neurite outgrowth. Etched circles may be washed 2 times with Hank's balanced salt solution plus calcium and magnesium (HBSS<sup>++</sup>) after the last rinse; 1.2 µl of L1-Fc (0.6µM, 0.3µM, 0.15 µM, 0.075 µM) is spotted onto the petri dishes to serve as a positive control and various concentrations of the L1-chondroitinase fusion protein can be applied to the plates as test samples. The plates can be incubated for 1 hour at room temperature. The spots are lightly aspirated, so as not to dry them out, and immediately washed 2 times with 1 ml HBSS<sup>++</sup> and then 1 ml of 1% BSA in PBS is added to each etched circle. After about 15 minutes at room temperature the etched circles can be washed 2 times with HBSS<sup>++</sup>, and once with bioassay medium (NeuralBasal(Gibco) + B27 supplements (Gibco) + L-glutamine + L- glutamic acid, penicillin and streptomycin (100 U/ml) + 1% fetal bovine serum which will remain on the petri dishes until the time of the assay. To provide neurons to plate on the substrates Cerebellar granule cells from postnatal day (PND) 9 or 10 are harvested. The brain is removed from the cranium of 1 pup, and the cerebellum separated from the rest of the tissue. Meninges is removed, and the cerebellum is placed in ice cold HBSS<sup>++</sup>. The tissue is minced and trypsinized using 0.25% trypsin for 15 minutes at 37°C. Trypsin action is inhibited by adding 0.5mg/ml soybean trypsin inhibitor (Gibco). The tissue is rinsed with HBSS<sup>++</sup> and triturated using a flame

narrowed Pasteur pipette coated with EBS. Dissociated cells are pelleted at 500xg for 3 minutes, the supernatant is decanted and the cells are resuspended in 2ml of growth medium. After lightly triturating, the cell suspension is carefully layered on top of a 3.5%BSA (in PBS) cushion, and spun at 500xg for 3 minutes. Supernatant is aspirated and the pellet is resuspended in 2 ml growth media. A cell count is performed and the cells are diluted to a final working concentration of  $1.5 \times 10^5$  cell/ml. A 300  $\mu$ l aliquot of diluted cells is added to the center of each plate, resulting in the even distribution of  $6 \times 10^4$  cells across the entire surface area of the etched circle. The cells are allowed to grow for 16 hours at 37°C in a humidified environment supplemented with 5% CO<sub>2</sub>. The next day, plates are removed from the 37°C incubator, cells are fixed with 4% paraformaldehyde and the outgrowth of neurites is recorded by photomicroscopy.

**[00114]** Biological activity assays for GGF2 may be performed using Schwann cell proliferation. Sciatic nerves are dissected from three day old Sprague Dawley rat pups and dissociated in L-15 medium (Invitrogen) containing 0.25% trypsin and 0.03% collagenase type I (SIGMA) for 15 minutes at 37°C. Nerves are centrifuged at 400xg for 5 minutes and dissociation medium is replaced by DMEM/10% FBS. Nerves are triturated using a 10 ml syringe with a 21g needle and subsequently a 23g needle. The cell suspension is filtered through a 40  $\mu$ m mesh placed over the opening of a 50ml conical tube. Cells can be centrifuged at 400xg for 5min, plated in a poly-D-Iysine (PDL)coated T-75 flask at a density of approximately 5 million cells in 15 ml of DMEM/10% FBS/PenStrep and incubated in a 37°C incubator regulated with 10% CO<sub>2</sub>. After a 24 hour incubation the cells can be washed twice with DMEM/10% FBS and re-fed with fibroblast inhibition medium consisting of DMEM/10%FBS and 10  $\mu$ l/ml of 1 mM cytosine arabinoside (Ara-C). After an incubation time of 2 to 3 days the fibroblast inhibition medium is replaced with Schwann cell growth medium (DMEM/10% FBS/ 150 ng/ml

of GGF2/5  $\mu$ M forskolin). Cells can be expanded and aliquots of  $2 \times 10^6$  cells/ml are frozen in DMEM/10%DMSO, 54% FBS in liquid nitrogen. At the time of use, Schwann cells are thawed and plated at a density of about 16,000 cells/well in a PDL-coated 96 well tissue culture plate in DMEM/5% FBS. After about 24 hours GGF2 is added in serial dilution ranging from 100 ng/ml to 0.78ng/ml containing 5  $\mu$ M forskolin to establish a standard curve. Samples of GGF2 fusion proteins can also be added in serial dilution together with 5  $\mu$ M forskolin. BrdU is added at 10  $\mu$ M. Cells are incubated for 48 hours in a 10% CO<sub>2</sub> incubator. A BrdU ELISA kit from Roche Applied Science can be used (Cat. No. 1 647 229) to detect Schwann cell proliferation. Medium is poured out of the plate and plate is tapped on tissue paper. A 200  $\mu$ l/well aliquot of Fix/Denat is added and incubated for 30 min at 15-25°C. A 100  $\mu$ l/well aliquot of anti-BrdU-POD working solution (lyophilized antibody is dissolved in 1.1 ml double distilled water and diluted 1:100 with antibody dilution solution) can be added and incubated for 90 min at room temperature. Wells may be washed 3 times with 200-300  $\mu$ l/well of washing solution. A 100  $\mu$ l aliquot of substrate solution is added and incubated for 15 minutes or until color development is sufficient for photometric detection. The Plates are read on a SpectraMax plate reader at 450nm either before addition of stop solution at 370nm or after addition of 50 ml 1N sulfuric acid to each well.

**[00115]** Akt [pS473] ELISA: C6 glioma cells, obtained from ATCC, are grown in DMEM/10%FBS in a T-75 flask to confluence. After trypsinization, cells are plated in a 24 well plate at a density of 500,000 cells/well in 0.5 ml of medium. One day after plating the cells are treated with GGF2 (batch: Glu from Lonza Biologics) at serial dilutions ranging from 0.78 to 100 ng/ml for 30 minutes at 37°C to establish a standard curve. As a negative control, wortmannin, a PI3-kinase inhibitor, is added to cells at 10 nM for 30 minutes before the

addition of GGF2. Samples of GGF2 fusion proteins will also be added in serial dilution. The cells are washed with PBS and then extracted with 100 $\mu$ l of cell extraction buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate, 1 mM PMSF, protease inhibitor cocktail (Pierce)). Cell extracts are kept at -80°C until further use. An ELISA kit from Biosource (Cat. #KHOO1 II) can be used to measure phosphorylated Akt kinase levels. Briefly, 100  $\mu$ l of a 1:50 dilution of samples and a serial dilution of Akt [pS473] standards are loaded into wells pre-coated with anti-Akt antibody for 2 h at room temperature (RT) or overnight at 4°C. Wells are washed and anti-Akt [pS473] antibody is added and incubated for 1 hour. After washing, HRP-conjugated anti-rabbit antiserum is added to wells and incubated for 30 min. After washing, TMB chromogen is added to wells and incubated for 30 min at RT before the reaction is stopped with stop solution. The plate is read on a SpectraMax plate reader at 450 nm. The optical densities are plotted against the Akt [pS473] standards and the concentration of pAkt in the C6 samples are deduced from the standard curve.

## EXAMPLE 6

**[00116]** This example illustrates construction of a fusion protein of chondroitinase polypeptide and a TAT cellular transduction peptide.

**[00117]** The gene sequence encoding a chondroitinase enzyme can be functionally linked to the protein transduction domain from HIV called the TAT Peptide. The resultant chimeric genes TAT-chondroitinase ABCI fusion DNA construct are shown in FIG. 1. It was observed that during bacterial expression of this construct, the TAT peptide was removed from

the chondroitinase enzyme at some processing point during the bacterial growth. The removal of n-terminal linked peptides was also observed during expression of an n-terminal histidine-tagged chondroitinase ABCI enzyme.

**[00118]** Deletion mutants of the chondroitinase ABCI enzyme were generated where the mutant was missing a certain number of amino acids from the n-terminal portion of the polypeptide but maintained its proteoglycan degrading activity. It was observed that these n-terminal deletion mutants maintained a histidine-tag that was attached to the n-terminus. It is expected that various TAT-deletion mutant chondroitinase ABCI fusion DNA constructs can be expressed without removal of the TAT polypeptide during expression. For example, the TAT peptide may be fused at the N-terminus of deletion mutants like ABCI-N $\Delta$ 20 or ABCI-N $\Delta$ 60 deletion mutant. Without wishing to be bound by theory, the inventors believe that the native proteoglycan degrading enzyme contains a signal sequence that is attached to the n-terminus. This signal sequence is removed during the natural production in bacteria and in production of the cloned enzyme in *E. coli*. It is thought that some signal within the n-terminal amino acids instructs the bacteria to remove anything attached to this end.

**[00119]** A DNA construct with the TAT-peptide attached to the N-terminus of one of the chondroitinase deletion mutants can be made and Western blot and protein gel showing this expressed protein and activity.

#### **EXAMPLE 7**

**[00120]** This example illustrates the diffusion of molecules into cells and tissue using a proteoglycan degrading composition.

**[00121]** A brain from an adult Sprague Dawley rat was removed from the skull and quartered into Right frontal, left frontal, right rear and left rear sections, corresponding roughly

to the frontal (front) and occipito-parietal (rear) lobes. (A) Right frontal quarter was placed in artificial cerebrospinal fluid (Catalog # 59-7316; Harvard Apparatus, Holliston, MA) containing the beta-galactosidase enzyme (Catalog #, G 5160; Sigma, St. Louis, MO) and chondroitinase ABC I at 0.5 U/ml (Catalog# C 3667; Sigma, St. Louis, MO) for 2 hours at 37°C. Brain quarter was rinsed several times in phosphate buffered saline (PBS) and then processed with a Beta-Gal staining kit (Catalog # Gal-S; Sigma, St. Louis, MO). The substrate-enzyme reaction (blue product) was allowed to develop for 1 hour, and the brain was rinsed several times in PBS and slabs from the middle of each brain block cut using parallel straight razors. (B) Left frontal quarter was placed in artificial cerebrospinal fluid (Catalog # 59-7316; Harvard Apparatus, Holliston, MA) containing the beta-galactosidase enzyme (Catalog #, G 5160; Sigma, St. Louis, MO) for 2 hours at 37°C. Brain quarter was rinsed several times in phosphate buffered saline (PBS) and then processed with a Beta-Gal staining kit (Catalog # Gal-S; Sigma, St. Louis, MO). The substrate-enzyme reaction (blue product) was allowed to develop for 1 hour, and the brain was rinsed several times in PBS and slabs from the middle of each brain block cut using parallel straight razors. (C) Right frontal quarter was placed in artificial cerebrospinal fluid (Catalog # 59-7316; Harvard Apparatus, Holliston, MA) containing the beta-galactosidase enzyme (Catalog #, G 5160; Sigma, St. Louis, MO) and chondroitinase ABC I at 0.5 U/ml (Catalog# C 3667; Sigma, St. Louis, MO) for 2 hours at 37°C. Brain quarter was rinsed several times in phosphate buffered saline (PBS) and then processed with a Beta-Gal staining kit (Catalog # Gal-S; Sigma, St. Louis, MO). The substrate-enzyme reaction (blue product) was allowed to develop for 1 hour, and the brain was rinsed several times in PBS and slabs from the middle of each brain block cut using parallel straight razors. (D) Left rear quarter was placed in artificial cerebrospinal fluid (Catalog # 59-7316; Harvard Apparatus, Holliston, MA) containing the beta-

galactosidase enzyme (Catalog #, G 5160; Sigma, St. Louis, MO) for 2 hours at 37°C. Brain quarter was rinsed several times in phosphate buffered saline (PBS) and then processed with a Beta-Gal staining kit (Catalog # Gal-S; Sigma, St. Louis, MO). The substrate-enzyme reaction (blue product) was allowed to develop for 1 hour, and the brain was rinsed several times in PBS and slabs from the middle of each brain block cut using parallel straight razors. Images of brains were acquired on a scanner and are shown in FIG. 2(I)(A-D).

**[00122]** Adult rat brain hemispheres were soaked in buffer alone or containing 33U/ml Chondroitinase ABCI (Acorda) for 2 hours at 37 degrees C. Hemispheres were rinsed and immediately placed in Eosin Y (Sigma) or a saturated solution of Congo Red (Sigma) in 70% ethanol. Slabs of tissue were cut and images were acquired on a scanner. See FIG. 2(II) Eosin and FIG. 2(III) Congo red.

**[00123]** FIG. 2(III) saturated solution of Congo Red demonstrates greater penetration through the cortex of a Chondroitinase treated brain hemisphere as compared to untreated brain. Congo Red is a negatively charged dye of 697 kDa. FIG. 2(II) Eosin Y penetration through the cortex of a Chondroitinase treated brain hemisphere looks slightly more diffuse, but penetration does not seem to be any deeper as compared to untreated brain. Eosin Y is zwitterionic, having an overall negative charged at the low pH it was used at, and it is 692 kDa. FIG. 2(I) Lobes from adult rat were incubated in beta-galactosidase alone (B&D), or with the addition of Chondroitinase ABCI (A, 0.5U/ml or C, 0.005U/m).

**[00124]** The results showed that chondroitinase treated tissue affected penetration of beta-galactosidase into CNS tissue. Chondroitinase had dramatic effects on the rate of Congo Red dye penetration but apparently not Eosin.



### EXAMPLE 8

**[00125]** This example illustrates a Chondroitinase ABCI Assay Protocol which may be modified to measure the activity of Chondroitinase ABCI deletion mutant-fusion proteins or the activity of other proteoglycan degrading polypeptide-fusion proteins of the present invention.

**[00126]** The production of reaction products from the catalytic activity of a proteoglycan degrading molecule or fusion protein is determined by a measurement of the absorbance of the product at a wavelength of 232 nm. A typical reaction mixture consisted of 120  $\mu$ l of reaction mixture (40mM Tris, pH 8.0, 40mM NaAcetate, 0.002% casein) combined with a substrate (5  $\mu$ l of 50 mM chondroitin C) and 1.5  $\mu$ l of chondroitinase ABCI or a fusion protein of a deletion mutant of chondroitinase ABCI-TAT. Seikagaku's cABCI reference: frozen at -20°C in 5ml aliquots, use 1ml per reaction, 50 mM chondroitin C (MW 521) in water: frozen at -20°C in 100ml aliquots. Reaction mixture aliquots of about 120  $\mu$ l can be prepared at 37°C for 3 min or longer. A wavelength of 232 nm, is used with the spectrometer.

**[00127]** Knowing the extinction coefficient for the reaction product, measuring the change in the absorbance of the reaction product at 232 nm reading over time upon addition of a known amount of the Chondroitinase ABCI or other other proteoglycan degrading polypeptide-fusion proteins to the 120  $\mu$ l reaction mixture with 0.002% casein and chondroitin substrate added, the specific activity in  $\mu$ mol/min/mg of the proteoglycan degrading fusion protein can be determined. Seikagaku Chondroitinase ABCI has a specific activity under these assay conditions of about 450  $\mu$ mole/min/mg.

**[00128]** Although the present invention has been described in considerable detail with reference to certain preferred embodiments thereof, other versions are possible. Therefore

the spirit and scope of the appended claims should not be limited to the description and the preferred versions contain within this specification.

## CLAIMS

What is claimed is:

1. A composition comprising:

an isolated nucleic acid comprising a sequence that encodes a polypeptide, said polypeptide comprising at least two of: a protein transduction domain, a proteoglycan degrading domain, or a domain that promotes neural regeneration.

2. The composition of claim 1 wherein nucleic acid encode for the protein transduction domain TAT domain.

3. The composition of claim 1 wherein the any two domains of said polypeptide are bonded together by a linker polypeptide domain.

4. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading domain that is a deletion mutant of chondroitinase ABCI.

5. The composition of claim 1 wherein the domain that promotes neural regeneration is L1, a variant that is at least 80% L1, or a deletion mutant of L1.

6. The composition of claim 1 wherein the domain that promotes neural regeneration is GGF2, a functional variant that is at least 80% GGF2, or a functional deletion mutant of GGF2.

7. The composition of claim 1 wherein the domain that promotes neural regeneration is NgR<sub>27-311</sub>, a functional variant that is at least 80% homologous with NgR<sub>27-311</sub>, or a functional deletion mutant of NgR<sub>27-311</sub>.

8. The composition of claim 1 expressed from genetically modified cells.

9. The composition of claim 1 and cells from the CNS.

10. A composition comprising:

a polypeptide comprising at least two of: a protein transduction domain, a proteoglycan degrading domain, or a domain that promotes neural regeneration.

11. The composition of claim 10 wherein the protein transduction domain is a TAT domain.
12. The composition of claim 10 wherein two domains of said polypeptide are bonded together by a linker polypeptide domain.
13. The composition of claim 10 wherein the proteoglycan degrading domain is a deletion mutant of chondroitinase ABCI.
14. The composition of claim 10 wherein the domain that promotes neural regeneration is L1, a variant that is at least 80% L1, or a deletion mutant of L1.
15. The composition of claim 10 wherein the domain that promotes neural regeneration is GGF2, a functional variant that is at least 80% GGF2, or a functional deletion mutant of GGF2.
16. The composition of claim 10 wherein the domain that promotes neural regeneration is NgR<sub>27-311</sub>, a functional variant that is at least 80% homologous with NgR<sub>27-311</sub>, or a functional deletion mutant of NgR<sub>27-311</sub>.
17. The composition of claim 10 having a chondroitinase.
18. The composition of claim 10 and a pharmaceutically acceptable excipient.
19. The composition of claim 10 and cells from the CNS.
20. A method of promoting recovery of neurological function comprising:
  - identifying a patient having comprised neurological function characterized by an SCI injury; and
  - administering a composition comprising at least two of: a protein transduction polypeptide, a proteoglycan degrading polypeptide, or a polypeptide that promotes neural regeneration. to a patient in need of such treatment.

21. The method of claim 20 wherein the composition is secreted by genetically modified cells that are implanted near the site of the CNS injury.
22. The composition of claim 20 wherein the proteoglycan degrading protein is Chondroitinase ABC I, Chondroitinase ABC II, Chondroitinase AC, Chondroitinase B, Hyaluronidase 1, Hyaluronidase 2, Hyaluronidase 3, Hyaluronidase 4, PH20, or combination of these.
23. The composition of claim 20 wherein the polypeptides are linked together.
24. A method of facilitating diffusion of an agent in a central nervous system comprising:  
  
administering the agent to the central nervous system; and  
  
modifying an extracellular matrix in the central nervous system to allow penetration of the agent into the extracellular matrix.
25. The method according to claim 24, further comprising the step of utilizing at least one enzyme capable of cleaving proteoglycans in the extracellular matrix.
26. The method according to claim 24, wherein the proteoglycans are chondroitin sulfate proteoglycans.
27. The method according to claim 24, wherein the at least one enzyme is selected from the group consisting of chondroitinase ABC Type I, chondroitinase ABC Type II, chondroitinase AC, chondroitinase B, hyaluronidase 1, hyaluronidase 2, hyaluronidase 3, hyaluronidase 4, cathepsins, ADAMT's, PH20, and any combination thereof.
28. The method according to claim 24, wherein the at least one enzyme is administered with one or more pharmaceutical carriers.
29. The method according to claim 24, wherein the agent is selected from the group consisting of complex biologics, small molecules, and transplanted cells.

30. The method according to claim 24, wherein administering the agent is accomplished topically, locally or systemically.
31. The method according to claim 24, wherein administering the agent is by bolus injection, intravenous delivery, continuous infusion, sustained release from implants, or sustained release pharmaceuticals.
32. A method of penetration of an agent through an extracellular matrix, comprising:  
  
administering at least one enzyme capable of cleaving chondroitin sulfate proteoglycans.
33. The method according to claim 32, wherein the at least one enzyme is selected from the group consisting of chondroitinase ABC Type I, chondroitinase ABC Type II, chondroitinase AC, chondroitinase B, hyaluronidase 1, hyaluronidase 2, hyaluronidase 3, hyaluronidase 4, cathepsins, ADAMT's, PH20, and combination thereof.
34. The method according to claim 32, wherein the at least one enzyme is administered in an effective amount.
35. The method according to claim 32, wherein the agent is selected from the group consisting of complex biologics, small molecules, and transplanted cells.
36. The method according to claim 32, further comprising the step of administering the agent topically, locally or systemically.
37. The method according to claim 32, further comprising the step of administering the agent by bolus injection, intravenous delivery, continuous infusion, sustained release from implants, or sustained release pharmaceuticals.
38. A method of enhancing uptake of an agent in a central nervous system, comprising:  
  
administering the agent and at least one enzyme capable of cleaving chondroitin sulfate proteoglycans.

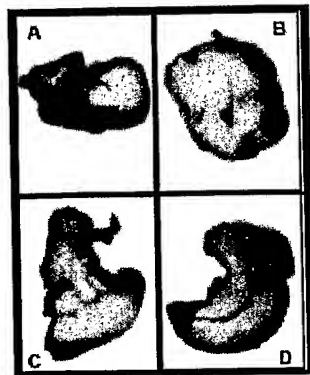
39. The method according to claim 38, wherein the at least one enzyme is selected from the group consisting of chondroitinase ABC Type I, chondroitinase ABC Type II, chondroitinase AC, chondroitinase B, hyaluronidase 1, hyaluronidase 2, hyaluronidase 3, hyaluronidase 4, cathepsins, ADAMT's, PH20, and any combination thereof.
40. The method according to claim 38, wherein the at least one enzyme is administered in an effective amount.
41. The method according to claim 38, wherein the at least one enzyme is administered with one or more pharmaceutical carriers.
42. The method of claim 38, wherein the pharmaceutical carrier is selected from the group consisting of diluents, binders, lubricants, coloring agents, disintegrating agents, buffer agents, isotonicizing agents, polyols, preservatives, and anesthetics.
43. The method according to claim 38, wherein the agent is selected from the group consisting of complex biologics, small molecules, and transplanted cells.
44. The method according to claim 38, wherein administering the agent is accomplished topically, locally or systemically.
45. The method according to claim 38, wherein administering the agent is by bolus injection, intravenous delivery, continuous infusion, sustained release from implants, or sustained release pharmaceuticals.

## TAT fusion chondroitinase ABCI nucleic acid

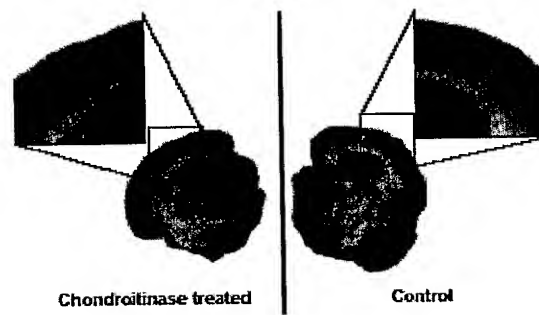
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FIG. 1

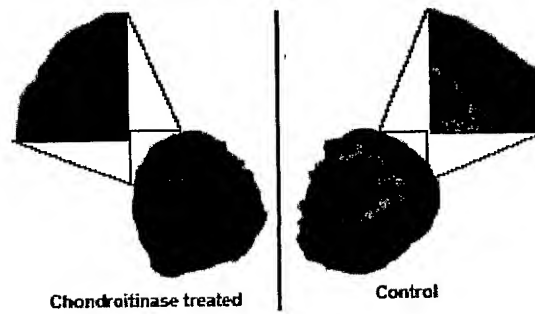




(I)

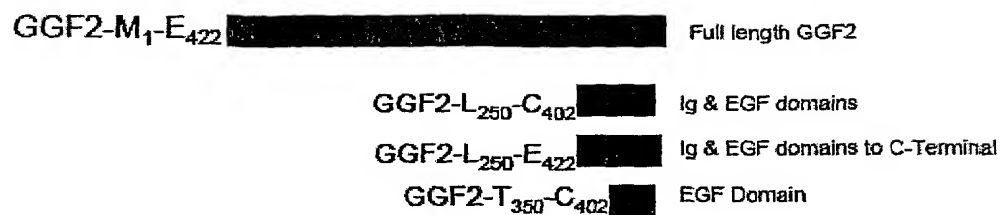


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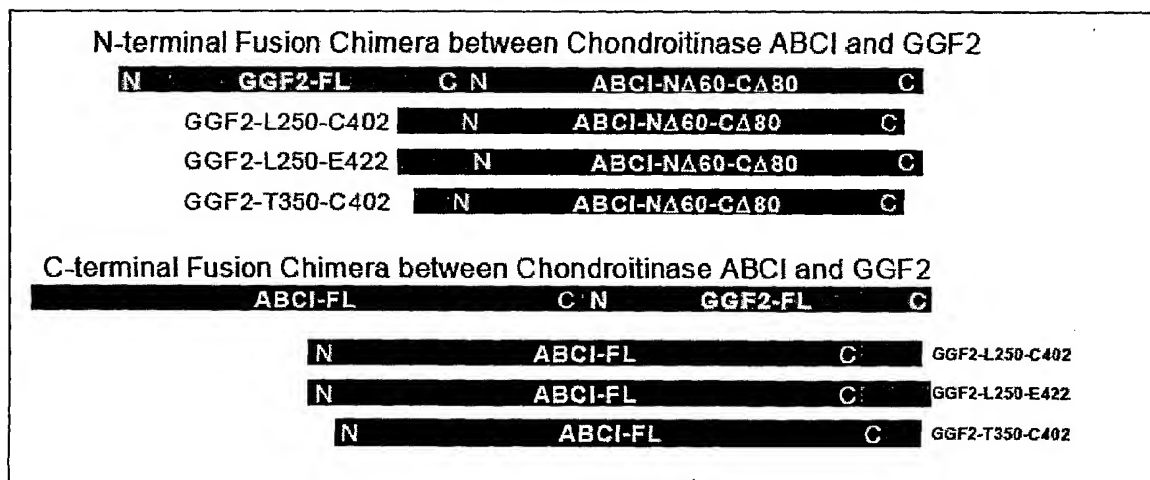


(III)

FIG. 2



(A)



(B)

FIG. 3

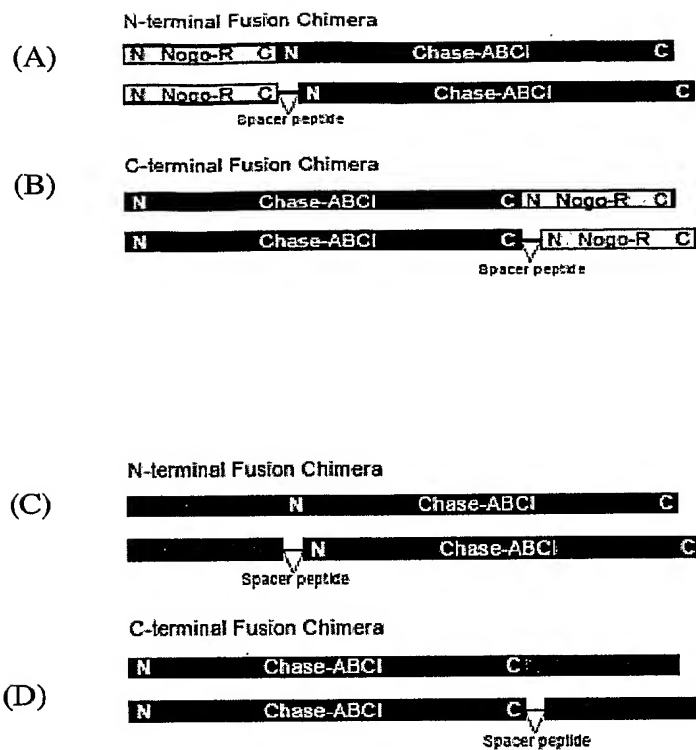


FIG. 4

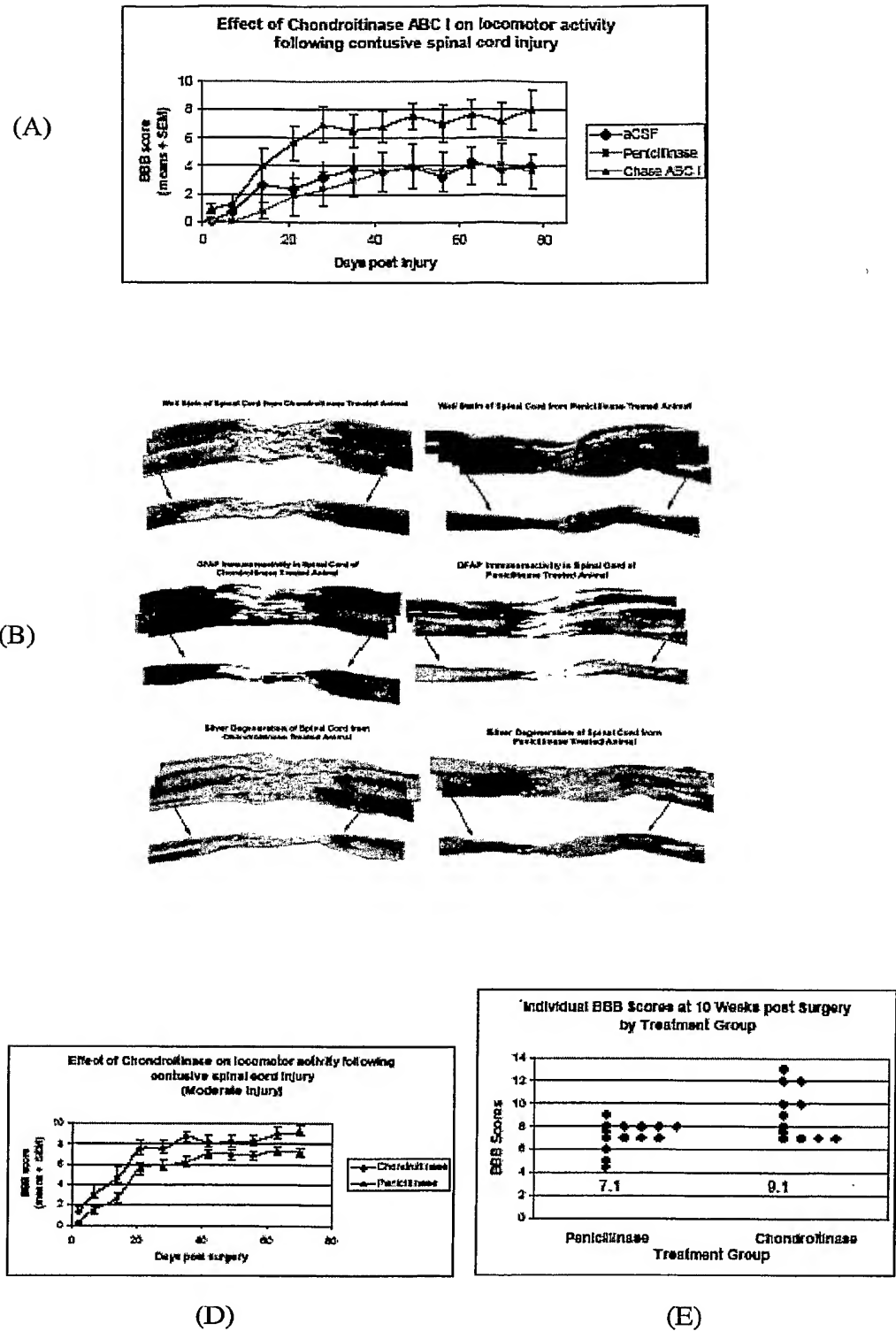


Figure 5

## SEQUENCE LISTING

SEQ ID NO: 1 Chondroitinase ABC I protein

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atsnpa fdpknlmqse iyhfaqnnpl adfssdknsi  
 61 ltlsdkrsim gnqslwkwk ggssftlhkk livptdkeas kawgrsstpv fsfwlynekp  
 121 idgyltidfg eklistsea agfkvkldft gwrTvgvsln ndlenremtl natntssdgt  
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepei qf  
 241 hnvkplpvt penlaaidli rqrlinefv geketnlale enisklksdf dalnThtlan  
 301 ggtqgrhlit dkqiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka  
 361 qlkqmyllmt khlldqgvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvysd  
 421 llwysrefks sfdmkvsads sdldyftls rqlhlallle pddqkrinlv ntfshtyga  
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk  
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk  
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr  
 661 ygryqshgva qivsngsqs qgyqqegwdw nrmegattih lplkdldspk phtlmqrger  
 721 gfsgtssleg qygmmafni ypanlerfdp nftakksvla adnhlifigs ninssdknkn  
 781 vetltfghai tptlntwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq  
 841 hqvsaenknr qptegnfsa widhstrpkd asyeymvfld atpekmngema qkfrenngly  
 901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvkn paivmthrqk dtlvisavtp  
 961 dlnmtrkaa tpvtinvtn gkwqsadkns evkyqvsgdn telftsifg ipqeiklspl  
 1021 p

SEQ ID NO: 2 NΔ20 ABCI (A<sub>45</sub>-N<sub>1023</sub>), protein

aqnnpl adfssdknsi  
 61 ltlsdkrsim gnqslwkwk ggssftlhkk livptdkeas kawgrsstpv fsfwlynekp  
 121 idgyltidfg eklistsea agfkvkldft gwrTvgvsln ndlenremtl natntssdgt  
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepei qf  
 241 hnvkplpvt penlaaidli rqrlinefv geketnlale enisklksdf dalnhtlan  
 301 ggtqgrhlit dkqiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka  
 361 qlkqmyllmt khlldqgvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvysd  
 421 llwysrefks sfdmkvsads sdldyftls rqlhlallle pddqkrinlv ntfshtyga  
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk  
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk  
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr  
 661 ygryqshgva qivsngsqs qgyqqegwdw nrmegattih lplkdldspk phtlmqrger  
 721 gfsgtssleg qygmmafni ypanlerfdp nftakksvla adnhlifigs ninssdknkn  
 781 vetltfghai tptlntwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq  
 841 hqvsaenknr qptegnfsa widhstrpkd asyeymvfld atpekmngema qkfrenngly  
 901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvkn paivmthrqk dtlvisavtp  
 961 dlnmtrkaa tpvtinvtn gkwqsadkns evkyqvsgdn telftsifg ipqeiklspl  
 1021 p

**SEQ ID NO: 3** NA60 ABCI (F<sub>85</sub>-N<sub>1023</sub>) protein

ftlhkk livptdkeas kawgrsstpv fsfwlynekp  
 121 idgyltidfg eklistseaq agfkvkl dft gwrvtgvsl n dlenremtl natntssdgt  
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepei qf  
 241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnthtlan  
 301 ggtqgrhlit dkqiiyqpe nl nsqdkqlf dnyvilgnyt tlmfnisray vlekdp tqka  
 361 qlkqmyllmt khlldqgvk gsalvtthw gyssrwwyis tlmsdalke anlqtqv yds  
 421 llwysrefks sfdmkvsads slddyfntls rqlal lllle pddqkrinlv ntfshyitga  
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgw nlnkk  
 541 amvsawiysn pev glplagr hpfnspslks vaqgyy wlam saksspdktl asiylaisdk  
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr  
 661 ygryqshgva qivsnsgsls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger  
 721 gfsgtssleg qygmmaf nli ypanlerfdp nftakksvla adnhlifigs ninssdknkn  
 781 vetltf qhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq  
 841 hqvsanenkr qptegnfsa widhstrpkd asyeymvfld atpekm gema qkfrenngly  
 901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk paivmthr qk dtlivsavtp  
 961 dlnmtrqkaa tptinv tin gkwqsadkns evkyqvsgdn tel tfts yfg ipqeikl spl  
 1021 p

**SEQ ID No. 4:** NA60 CA80 ABCI (F<sub>85</sub>-A<sub>942</sub>) protein

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 121 idgyltidfg eklistseaq agfkvkl dft gwrvtgvsl n dlenremtl natntssdgt  
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepei qf  
 241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnthtlan  
 301 ggtqgrhlit dkqiiyqpe nl nsqdkqlf dnyvilgnyt tlmfnisray vlekdp tqka  
 361 qlkqmyllmt khlldqgvk gsalvtthw gyssrwwyis tlmsdalke anlqtqv yds  
 421 llwysrefks sfdmkvsads slddyfntls rqlal lllle pddqkrinlv ntfshyitga  
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 781 vetltf qhai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq  
 841 hqvsanenkr qptegnfsa widhstrpkd asyeymvfld atpekm gema qkfrenngly  
 901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk pa

**SEQ ID NO: 5** Chondroitinase AC protein Locus 1HMW\_A**ORIGIN**

1 mkklfvtciv ffsilspall iaqqtga el imkrvmldlk kplrnmdkva eknltlqpd  
 61 gswkdvp ykd damtnwlpnn hllqletii q ayiekdshyy gddkvfdqis kafkywydsd  
 121 pksrnwwhne iatpqalgem lilmrygkpp lde alvhklt ermkrgepek ktganktdia  
 181 lhyfy rallt sdeallsfav kelfypvqfv hyeeglqdy sylqhgpqlq issygavfit  
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka  
 301 ekkrl lvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv  
 361 rmvskrttrs esgnkenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd  
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna  
 481 penittlnq swl ngpvist agktgrgkit tfkaaggqfwl lhdaigyyfp eganlslstq

541 sqkgnwfhin nshskdevsg dvfklwinhg arpenaqyay ivlpginkpe eikkyngtap  
 601 kvlantnqlq avyhhqldmv qaifytagkl svagieietd kpcavlikhi ngkqviwaad  
 661 plqkektavl sirdlktgkt nrvkidfpqq efagatvelk

**SEQ ID NO: 6** CA200 AC (Q<sub>23</sub>-T<sub>500</sub>) protein

qqtgtael imkrvmldlk kplrnmdkva eknlnlqpd  
 61 gswkdvpkyd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd  
 121 pksrnwwhne iatpqalgem lilmrygkpk ldealvhklt ermkrgepek ktganktdia  
 181 lhyfyralit sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit  
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka  
 301 ekkrlivakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv  
 361 rmvsktrtrs esgngenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd  
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna  
 481 penittlnq swlmgpvist

**SEQ ID NO: 7** CA220 AC (Q<sub>23</sub>-A<sub>480</sub>) protein

qqtgtael imkrvmldlk kplrnmdkva eknlnlqpd  
 61 gswkdvpkyd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd  
 121 pksrnwwhne iatpqalgem lilmrygkpk ldealvhklt ermkrgepek ktganktdia  
 181 lhyfyralit sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit  
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka  
 301 ekkrlivakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv  
 361 rmvsktrtrs esgngenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd  
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna

**SEQ ID NO: 8** NA20 CA200 AC (L<sub>43</sub>-T<sub>500</sub>) protein

lrmmdkva eknlnlqpd  
 61 gswkdvpkyd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd  
 121 pksrnwwhne iatpqalgem lilmrygkpk ldealvhklt ermkrgepek ktganktdia  
 181 lhyfyralit sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit  
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka  
 301 ekkrlivakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv  
 361 rmvsktrtrs esgngenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd  
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna  
 481 penittlnq swlmgpvist

**SEQ ID NO: 9** NA50 CA200 AC (T<sub>74</sub>-T<sub>500</sub>) protein

tnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd  
 121 pksrnwwhne iatpqalgem lilmrygkpk ldealvhklt ermkrgepek ktganktdia  
 181 lhyfyralit sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit  
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka  
 301 ekkrlivakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv  
 361 rmvsktrtrs esgngenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd  
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna  
 481 penittlnq swlmgpvist

**SEQ ID NO: 10** NA100 CA200 AC (S<sub>123</sub>-T<sub>500</sub>) protein

srnwwhne iatpqalgem lilmrygkpk ldeavhkl ermkrgepek ktganktdia  
 181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgplq issygavfit  
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka  
 301 ekkrlivakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv  
 361 rmvsktrrs esgkenllg rylsdgatni qlrgpeyyi mpvwewdkip gitsrdyltd  
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna  
 481 penittlnq swlmgpvist

**SEQ ID NO: 11** NA50 CA275 AC (T<sub>74</sub>-L<sub>426</sub>) protein

tnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd  
 121 pksrnwwhne iatpqalgem lilmrygkpk ldeavhkl ermkrgepek ktganktdia  
 181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgplq issygavfit  
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka  
 301 ekkrlivakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv  
 361 rmvsktrrs esgkenllg rylsdgatni qlrgpeyyi mpvwewdkip gitsrdyltd  
 421 rpltkl

**SEQ ID NO: 12** Chondroitinase B Locus Q46079 protein**ORIGIN**

1 mkmlnklagy llpimvllnv apclgqvvas netlyqvve vkpgglvqia dgtykdvqli  
 61 vsnsgkslpl itikalnpgk vfftdakve lrgehlileg iwfkdgndrai qawkshgpgl  
 121 vaiygsynri tacvfcdfe ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln  
 181 ntaraidgs vggpgmyhrv dhcfsnpqk pgnagggi gyyrndigrc lvdsnlfmrq  
 241 dseaeiitsk sqenvyygnt ylnqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs  
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp  
 361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve  
 421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgkplswdev rpywlkempg  
 481 tyaltarlisa draakfkavi krnkeh

**SEQ ID NO: 13** NA80 Chase B (G<sub>106</sub>-H<sub>506</sub>) protein

gnrai qawkshgpgl  
 121 vaiygsynri tacvfcdfe ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln  
 181 ntaraidgs vggpgmyhrv dhcfsnpqk pgnagggi gyyrndigrc lvdsnlfmrq  
 241 dseaeiitsk sqenvyygnt ylnqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs  
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp  
 361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve  
 421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgkplswdev rpywlkempg  
 481 tyaltarlisa draakfkavi krnkeh

**SEQ ID NO: 14** NA120 Chase B (I<sub>146</sub>-H<sub>506</sub>) protein

ittsl tedgkvpqhc ridhcsftdk itfdqvinln  
 181 ntaraidgs vggpgmyhrv dhcfsnpqk pgnagggi gyyrndigrc lvdsnlfmrq  
 241 dseaeiitsk sqenvyygnt ylnqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs  
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp



361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve  
 421 kgipvnisan rsaykpvkik diqpiegial dlaliskgi tgkplswdev rpywlkempg  
 481 tyaltarlsa draakfkavi krnkeh

**SEQ ID NO: 15** CA19 Chase B (Q<sub>26</sub>-L<sub>488</sub>) protein

qvvas netlyqvcke vkpgglvqia dgtykdvqli  
 61 vsnsgksglp itikalnpgk vfftdakve lrgehlileg iwfkdgndrai qawkshgpgl  
 121 vaiygsynri tacvfcdede ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln  
 181 ntaraidgs vggpgmyhrv dhcfsnpqk pgnagggi ggyrndigrc lvdsnlfmrq  
 241 dseaeiitsk sqenvyygnt ylnccgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs  
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngyaihfnpl  
 361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve  
 421 kgipvnisan rsaykpvkik diqpiegial dlaliskgi tgkplswdev rpywlkempg  
 481 tyaltarl

**SEQ ID NO: 16** CA120 Chase B (Q<sub>26</sub>-K<sub>390</sub>) protein

qvvas netlyqvcke vkpgglvqia dgtykdvqli  
 61 vsnsgksglp itikalnpgk vfftdakve lrgehlileg iwfkdgndrai qawkshgpgl  
 121 vaiygsynri tacvfcdede ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln  
 181 ntaraidgs vggpgmyhrv dhcfsnpqk pgnagggi ggyrndigrc lvdsnlfmrq  
 241 dseaeiitsk sqenvyygnt ylnccgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs  
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngyaihfnpl  
 361 derrkeycaa nrlkfetphq lmlkgnlffk

**SEQ ID NO: 17** NA120 CA120 Chase B (I<sub>146</sub>-K<sub>390</sub>) protein

ittsl tedgkvpqhc ridhcsftdk itfdqvinln  
 181 ntaraidgs vggpgmyhrv dhcfsnpqk pgnagggi ggyrndigrc lvdsnlfmrq  
 241 dseaeiitsk sqenvyygnt ylnccgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs  
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngyaihfnpl  
 361 derrkeycaa nrlkfetphq lmlkgnlffk

**SEQ ID NO: 18** Choindroitinase AC nucleotide locus CHU27583

**ORIGIN**

1 atgaagaaat tatttgtaac ctgtatagtc ttttctcta ttttaagtc tgctctgctt  
 61 attgcacagc agaccggtagc gcagaactg attatgaagc gggtagtgct ggaccttaaa  
 121 aagcctttgc gcaatatgga taaggtggcg gaaaagaacc tgaatacgt gcagcctgac  
 181 ggtagctgga aggatgtgcc ttataaagat gatgccatga ccaattggtt gccaaacaac  
 241 cacctgctac aattggaaac tattatacag gcttatattg aaaaagatag tcaactattat  
 301 ggcgacgata aagtgtttga ccagatttcc aaagcttita agtattggta tgacagcgac  
 361 ccgaaaagcc gcaactggtag gcacaatgaa attgccactc cgcaggccct tggtagaatg  
 421 ctgatctga tgcgttacgg taaaaagccg ctgtatgaag cattggtagc taaattgacc  
 481 gaaagaatga agcggggcgga accgggagaag aaaacggggg ccaacaaaac agatcgcgc  
 541 ctgcattact ttatcgtgc ttgttaacg tctgatgagg ctttgcttcc cttcgccgta  
 601 aaagaattgt ttatcccgat acagtttgta cactatgagg aaggcctgca atacgattat  
 661 tcctacctgc agcacggtagc gcaattacag atatcgagct acggtagcgt attattacc  
 721 ggggtactga aacttgccaa ttacgttagg gataccctt atgctttaag taccgagaaa  
 781 ctggctatat tttaaaagta ttaccgcgac agttatctga aagctatccg tggaagtat

841 atggatttta acgtagaagg ccgcggagta agccggccag acattctaaa taaaaaggca  
 901 gaaaaaaaga gggtgctggt ggccaagatg atcgatctta agcactactga agaattgggt  
 961 gatgcgatag ccaggacaga tagcacagtt gcggccggct ataagattga gccctatcac  
 1021 catcagttct ggaatggtga ttatgtgcaa catttaagac ctgcctattc ttttaattgt  
 1081 cgtatggtga gtaagcggac ccgacgcagt gaatccggca ataaagaaaa cctgctgggc  
 1141 aggtatttat ctgatggggc tactaacata caattgcgcg gaccagaata ctataacatt  
 1201 atgccggtat gggaatggga caagattcct ggcataacca gccgtgatta ttaaccgac  
 1261 agacctttga cgaagctttg gggagagcag gggagcaatg actttgcagg aggggtgtct  
 1321 gatggtgtat acggggccag tgcctacgca ttggattacg atagcttaca ggcaaaagaa  
 1381 gcctggttct ttttgacaa agagattgta tgccttggtg ccggtatcaa cagcaatgcc  
 1441 cctgaaaaca ttaccactac ccttaaccag agctggttaa atggcccggt tataagtact  
 1501 gcaggtaaaa ccggccgggg taaaataaca acgtttaag cacagggaca gttctggttg  
 1561 ttgcacgatg cgattggtta ttactttcct gaagggggcca accttagtct gagtaccag  
 1621 tcgcaaaaag gcaattggtt ccacatcaac aattcacatt caaaagatga agtttctggt  
 1681 gatgtattta agctttggat caaccatggt gccaggccag aaaatgcgca gtatgcttat  
 1741 atcgttttgc cggaataaaa caagccggaa gaaattaaaa aatataatgg aacggcaccg  
 1801 aaagtccttg ccaataccaa ccagctgcag gcagttatc atcagcagtt agatatggt  
 1861 caggctatct tctatacagc tggaaaatta agcgtagcgg gcatagaaat tgaaacagat  
 1921 aagccatgtg cagtgtgat caagcacatc aatggcaagc aggttaattg ggctgccgat  
 1981 ccattgcaaa aagaaaagac tgcagtgttg agcatcaggg atttaaaaac aggaaaaaca  
 2041 aatcgggtaa aaattgattt tccgaacag gaatttcag gtgcaacggt tgaactgaaa  
 2101 tag

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**SEQ ID NO: 19** Chondriotinase AC nucleic acid deletion NΔ50 C Δ275 (a<sub>220</sub> – t<sub>1278</sub>)

atgccatga ccaattggtt gccaaacaac

241 cacctgtac aattggaac tattatacag gcttatattg aaaaagatag tctactattat  
 301 ggcgacgata aagtgttga ccagatttcc aaagctttta agtattggtg tgacagcgac  
 361 ccgaaaagcc gcaactggtg gcacaatgaa attgccactc cgcaggccct tggtagaatg  
 421 ctgatcctga tgcgttacgg taaaaagccg cttgatgaag cattggtgca taaattgacc  
 481 gaaagaatga agcggggcga accggagaag aaaacggggg ccaacaaaac agatcgcg  
 541 ctgcattact tttatcgtgc ttgttaacg tctgatgagg ctttgcttcc ctccgccgta  
 601 aaagaattgt tttatcccgt acagittgta cactatgagg aaggcctgca atacgattat  
 661 tctacctgc agcacgggcc gcaattacag atatcgagct acggtgccgt atttattacc  
 721 ggggtactga aacttgccaa ttacgttagg gatacccctt atgctttaag taccgagaaa  
 781 ctggctatat ttcaaagta ttaccgcgac agttatctga aagctatccg tggaagtat  
 841 atggatttta acgtagaagg ccgcggagta agccggccag acattctaaa taaaaaggca  
 901 gaaaaaaaga gggtgctggt ggccaagatg atcgatctta agcactactga agaattgggt  
 961 gatgcgatag ccaggacaga tagcacagtt gcggccggct ataagattga gccctatcac  
 1021 catcagttct ggaatggtga ttatgtgcaa catttaagac ctgcctattc ttttaattgt  
 1081 cgtatggtga gtaagcggac ccgacgcagt gaatccggca ataaagaaaa cctgctgggc  
 1141 aggtatttat ctgatggggc tactaacata caattgcgcg gaccagaata ctataacatt  
 1201 atgccggtat gggaatggga caagattcct ggcataacca gccgtgatta ttaaccgac  
 1261 agacctttga cgaagctt

**SEQ ID NO: 20** Chondroitinase B nuclei acid Locus CHU27584

ORIGIN

1 atgaagatgc tgaataaact agccggatac ttattgccga tcatggtgct gctgaatgtg  
 61 gcaccatgct taggtcaggt tgttgcttca aatgaaactt tataccaggt tgtaaaggag

121 gtaaaacccg gtggtctggt acagattgcc gatgggactt ataaagatgt tcagctgatt  
 181 gtcagcaatt caggaaaatc tggttgccc atcactatta aagccctgaa cccgggtaag  
 241 gttttttta cgggagatgc taaagtagag ctgaggggagc agcacctgat actggaaggc  
 301 atctggttta aagacgggaa cagagctatt caggcatgga aatcacatgg acccggattg  
 361 gtggctatat atggtagcta taaccgcatt accgcatgtg tattgattg tttgatgaa  
 421 gccaatctg cttacattac tacttcgctt accgaagacg gaaaggtacc tcaacattgc  
 481 cgcatagacc attgcagttt taccgataag atcactttg accaggtaat taacctgaac  
 541 aatacagcca gagctattaa agacgggtcg gtgggaggac cggggatgta ccatcgtgtt  
 601 gatcactgtt tttttccaa tccgcaaaaa cgggtaatg cgggaggggg aatcaggatt  
 661 ggctattacc gtaatgatat aggccgttgt ctggtagact ctaacctgtt tatgcgtcag  
 721 gattcggaag cagagatcat caccagcaaa tcgcaggaaa atgtttatta tggtaatact  
 781 tacctgaatt gccagggcac catgaacttt cgtcacgggt atcatcaggt ggccattaac  
 841 aattttata taggcaatga ccagcgattt ggatacgggg gaatgttgt ttggggaagc  
 901 aggcattgca tagcctgtaa ttatttgag ctgtccgaaa ccataaagtc gagggggaac  
 961 gccgcattgt attaaaccc cgggtctatg gcttcggagc atgctctgc tticgatatg  
 1021 ttgatagcca acaacgctt catcaatgta aatgggtatg ccatccattt taatccattg  
 1081 gatgagcgca gaaaagaata ttgtgcagcc aataggctta agttcgaac cccgcaccag  
 1141 ctaatgttaa aaggcaatct tttcttaag gataaacctt atgtttacc attttttaa  
 1201 gatgattatt ttatagcagg gaaaaatagc tggactggtat atgtaccctt aggtgtggaa  
 1261 aagggaatcc ctgttaacat ttggccaat aggtctgcct ataagccggt aaaaatttaa  
 1321 gatatccagc ccatagaagg aatcgctctt gatctcaatg cgtgatcag caaaggcatt  
 1381 acaggaaagc cccttagctg ggatgaagta aggcctact ggtaaaaga aatgccggg  
 1441 acgtatgctt taacggccag gcttctgca gatagggctg caaagtttaa agccgtaatt  
 1501 aaaagaaata aagagcactg a

**SEQ ID NO: 21** Chondriotinase B nucleic acid deletion NΔ120 CΔ120 (a<sub>436</sub> – g<sub>1170</sub>)

attac tacttcgctt accgaagacg gaaaggtacc tcaacattgc  
 481 cgcatagacc attgcagttt taccgataag atcactttg accaggtaat taacctgaac  
 541 aatacagcca gagctattaa agacgggtcg gtgggaggac cggggatgta ccatcgtgtt  
 601 gatcactgtt tttttccaa tccgcaaaaa cgggtaatg cgggaggggg aatcaggatt  
 661 ggctattacc gtaatgatat aggccgttgt ctggtagact ctaacctgtt tatgcgtcag  
 721 gattcggaag cagagatcat caccagcaaa tcgcaggaaa atgtttatta tggtaatact  
 781 tacctgaatt gccagggcac catgaacttt cgtcacgggt atcatcaggt ggccattaac  
 841 aattttata taggcaatga ccagcgattt ggatacgggg gaatgttgt ttggggaagc  
 901 aggcattgca tagcctgtaa ttatttgag ctgtccgaaa ccataaagtc gagggggaac  
 961 gccgcattgt attaaaccc cgggtctatg gcttcggagc atgctctgc tticgatatg  
 1021 ttgatagcca acaacgctt catcaatgta aatgggtatg ccatccattt taatccattg  
 1081 gatgagcgca gaaaagaata ttgtgcagcc aataggctta agttcgaac cccgcaccag  
 1141 ctaatgttaa aaggcaatct tttcttaag

**SEQ ID NO: 22** Chondroitinase ABCI nucleic acid Locus I29953

**ORIGIN**

1 ggaattccat cactcaatca ttaaatttag gcacaacgat gggctatcag cgttatgaca  
 61 aatttaatga aggacgcatt ggtttactg ttagccagcg ttctaagga gaaaaataat  
 121 gccgatattt cgtttactg cacttgcaat gacattgggg ctattatcag cgccttataa  
 181 cgcatgggca gccaccagca atcctgcatt tgatcctaaa aatctgatgc agtcagaaat  
 241 ttaccatttt gcacaaaata acccattagc agacttctca tcagataaaa actcaatact  
 301 aacgttatct gataaacgta gcattatggg aaaccaatct cttttatgga aatggaaagg

361 tggtagtagc ttactttac ataaaaaact gattgtcccc accgataaag aagcatctaa  
421 agcatgggga cgctcatcta cccccgttt ctcattttgg cttfacaatg aaaaaccgat  
481 tgatggttat ctactatcg atttcggaga aaaactcatt tcaaccagtg aggctcaggc  
541 aggcctttaa gtaaaatag atttactgg ctggcgtgct gtgggagtct ctttaataa  
601 cgatcttgaa aatcgagaga tgacctaaa tgcaaccaat acctcctctg atggtactca  
661 agacagcatt gggcgttct taggtgctaa agtcgatagt attcgttta aagcgccttc  
721 taatgtgagt cagggtgaaa tctatatcga ccgtattatg tttctgtcg atgatgctcg  
781 ctaccaatgg tctgattatc aagtaaaaac tcgcttatca gaacctgaaa tcaatttca  
841 caacgtaaag ccacaactac ctgtaacacc tgaaaattta gcggccattg atcttattcg  
901 ccaacgtcta attaataat ttgtcggagg tgaaaagag acaaacctcg cattagaaga  
961 gaatatcagc aaattaaaaa gtgatttca tgctcttaat attcacact tagcaaatgg  
1021 tggaacgcaa ggcagacatc tgatcactga taaacaaatc attattatc aaccagagaa  
1081 tcttaactcc caagataaac aactatttga taattatgtt attttaggta attacacgac  
1141 attaatgttt aatattagcc gtgcttatgt gctggaaaaa gatccacac aaaaggcgca  
1201 actaaagcag atgtacttat taatgacaaa gcatttatta gatcaaggct ttgttaaagg  
1261 gagtgttita gtgacaacc atcactgggg atacagtct cgttggtgtt atattccac  
1321 gttattaatg tctgatgcac taaaagaagc gaacctaca actcaagtt atgattcatt  
1381 actgtggtat tcactgtagt taaaagtag tttgatatg aaagtaagt ctgatagctc  
1441 tgatctagat tattcaata ccttatctcg ccaacattt gccttatt tactagagcc  
1501 tgatgatcaa aagcgatca acttagttaa tacttcagc cattatatca ctggcgcat  
1561 aacgcaagt ccaccgggtg gtaaagatgg ttacgccct gatggtacag catggcgaca  
1621 tgaaggcaac tatccgggt actctttccc agcctttaa aatgcctctc agcttatta  
1681 ttattacgc gataccat ttcagtggg tgaaagtgt tggaataacc tgaaaaagc  
1741 gatgtttca gcgtgatct acagtaatcc agaagtggga ttaccgctt caggaagaca  
1801 ccttttaac tcacctctg taaaatcagt cgctcaaggc tattactggc ttgcatgct  
1861 tgcaaaatca tcgctgata aaacactgc atctattat ctgctgatta gtgataaac  
1921 acaaaatgaa tcaactgcta ttttggaga aactattaca ccagcgtct tactcaagg  
1981 tttctatgcc ttaatggcg gtgcttttg tattcatctg tggcaagata aaatggtgac  
2041 actgaaagt tataacacca atgttggtc atctgaaatt tataacaaag ataaccgtta  
2101 tggccgtac caaagtcag gtgtcgtca aatagttagt aatggctgc agcttcaca  
2161 gggctatcag caagaaggt gggattggaa tagaatgcaa ggggcaacca ctattcacct  
2221 tcctcttaa gacttagaca gtctaaacc tcataccta atgcaacgtg gagagcgtgg  
2281 atttagcga acatcatccc tgaaggta atatggcatg atggcattc atcttatta  
2341 tcccgcaat ctgagcgtt ttgacctaa ttactgctg aaaaagagt tattagccgc  
2401 tgataatcac ttaatttta ttgtagcaa tataaatagt agtgataaaa ataaaaatg  
2461 tgaaacgacc ttattcaac atgccattac tcaacatta aataccctt ggattaatg  
2521 acaaaagata gaaaacatgc ctatcaaac aacacttcaa caaggtgatt ggtaattga  
2581 tagcaatggc aatggttact taattactca agcagaaaaa gtaaatgtaa gtcgccaaca  
2641 tcaggtttca gcggaaaata aaaatcgcca accgacagaa ggaaactta gtcggcatg  
2701 gatcgatcac agcactgcc ccaaagatgc cagttagag tatatggtct ttttagatgc  
2761 gacacctgaa aaaatgggag agatggcaca aaaattccgt gaaaataatg gggtatatca  
2821 ggttctctg aaggataaag acgttcatat tattctgat aaactcagca atgtaacggg  
2881 atatgcctt tatcagccag catcaattga agacaaatgg atcaaaaagg ttaataaacc  
2941 tgcaattgtg atgactatc gacaaaaa cactcttatt gtcagtgcag ttacacctga  
3001 tttaaatatg actcgccaaa aagcagcaac tcctgtcacc atcaatgtca cgattaatg  
3061 caaatggcaa tctgctgata aaaatagtga agtgaaatat caggtttctg gtgataaac  
3121 tgaactgacg ttacgagtt actttggtat tccacaagaa atcaactct cgccactccc  
3181 ttgattaat caaagaacg ctctgcgtt cctttttat ttgcaggaaa tctgattatg  
3241 ctaataaaaa accctttagc ccacgcggtt acattaagcc tctgtttatc attaccgca  
3301 caagcattac ccactctgc tcatgaagct ttgcgcgata ttatcttt tgaaagtgaa

3361 ttaccaata cccttaccac ttcaataat aatcaattat cgctaagcaa acagcatgct  
 3421 aaagatgggtg aacaatcact caaatggcaa tatcaaccac aagcaacatt aacactaaat  
 3481 aatattgtta attaccaaga tgataaaaat acagccacac cactcacttt tatgatgtgg  
 3541 atttataatg aaaaaccica atcttcccca ttaacgttag catttaaaca aaataataaa  
 3601 attgactaa gttttaatgc tgaacttaat ttacgggggt ggcgaggtat tgctgttcct  
 3661 ttctgtgata tgcaaggctc tgcgacaggt caacttgatc aattagtgat caccgctcca  
 3721 aaccaagccg gaacactctt ttttgatcaa atcatcatga gtgtaccgtt agacaatcgt  
 3781 tgggcagtac ctgactatca aacaccttac gtaataacg cagtaaacac gatggtagt  
 3841 aaaaactgga gtgcattatt gatgtacgat cagatgttc aagcccatta cctacttta  
 3901 aacttcgata ctgaatttcg cgatgaccaa acagaaatgg cticgattta tcagcgcttt  
 3961 gaattatc aaggaaattcc

//

**SEQ ID NO: 23** TAT fusion chondroitinase ABCI nucleic acid

ggctgtaaaaagcgtcgtcaacgtcgtc  
 tggtgggtgggtgggtgccaccagca atcctgcatt tgatcctaaa aatctgatgc agtcagaaat  
 241 ttaccatttt gcacaaaata acccattagc agacttctca tcagataaaa actcaatact  
 301 aacgttatct gataaacgta gcattatggg aaaccaatct cttttatgga aatggaaagg  
 361 tggtagtagc ttactttac ataaaaaact gattgtcccc accgataaag aagcatctaa  
 421 agcatgggga cgctcatcta ccccgtttt ctcatittgg ctttacaatg aaaaaccgat  
 481 tgatggttat ctactatcg atttcggaga aaaactcatt tcaaccagtg aggctcaggc  
 541 aggcctttaa gtaaaattag atttactgg ctggcgtgct gtgggagtct ctttaataa  
 601 cgatcttgaa aatcgagaga tgaccttaa tgcaaccaat acctcctctg atggtactca  
 661 agacagcatt gggcgttctt taggtgctaa agtcgatagt attcgttta aagcgcttc  
 721 taatgtgagt cagggtgaaa tctatatcga ccgtattatg tttctgtcg atgatgctc  
 781 ctaccaatgg tctgattatc aagtaaaaac tcgcttatca gaacctgaaa ttcaattca  
 841 caacgtaaag ccacaactac ctgtaacacc tgaaaattta gcggccattg atcttattcg  
 901 ccaacgtcta attaatgaat ttgtcggagg tgaaaaagag acaaacctcg cattagaaga  
 961 gaatatcagc aaattaaaaa gtgatttca tgctcttaat attcacactt tagcaaatgg  
 1021 tggaacgcaa ggcagacatc tgatcactga taaacaaatc attattatc aaccagagaa  
 1081 tcttaactcc caagataaac aactatttga taattatgtt attttaggta attacacgac  
 1141 attaatgttt aatattagcc gtgcttatgt gctggaaaaa gatccacac aaaaggcgca  
 1201 actaaagcag atgtacttat taatgacaaa gcatttatta gatcaaggct tigttaaagg  
 1261 gagtgcttta gtgacaaccc atcactgggg atacagtct cgttgggtgt atattccac  
 1321 gttattaatg tctgatgcac taaaagaagc gaacctaca actcaagttt atgattcatt  
 1381 actgtggtat tcacgtgagt ttaaaagtag tttgatatg aaagtaagtg ctgatagtc  
 1441 tgatctagat tatttcaata ccttatctcg ccaacattta gccttattat tactagagcc  
 1501 tgatgatcaa aagcgtatca acttagttaa tactttcagc cattatatca ctggcgcat  
 1561 aacgcaagtg ccaccgggtg gtaaagatgg ttacgcctt gatggtacag catggcgaca  
 1621 tgaaggcaac tatccgggct actctttccc agcctttaa aatgcctctc agcttattta  
 1681 ttattacgc gataccatc ttctagtggg tgaaagtggg tggaataacc tgaaaaaagc  
 1741 gatggtttca gcgtggatct acagtaatcc agaagtggg ttaccgcttg caggaagaca  
 1801 ccttttaac tcaccttctg taaaatcagt cgctcaaggc tattactggc ttgccatgc  
 1861 tgcaaaatca tcgctgata aaacacttgc atctatttat ctgcgatta gtgataaac  
 1921 acaaaatgaa tcaactgcta ttttggaga aactattaca ccagcgtctt tacctcaagg  
 1981 tttctatgcc tttatggcg gtgcttttgg tattcatcgt tggcaagata aaatgggtgac  
 2041 actgaaagct tataacacca atgtttggc atctgaaatt tataacaaag ataaccgtta  
 2101 tggccgttac caaagtcag gtgtcgtca aatagtgagt aatggctcgc agctttcaca  
 2161 gggctatcag caagaagggt gggattggaa tagaatgcaa ggggcaacca ctattcacct  
 2221 tctcttaaa gacttagaca gtcctaaacc tcatacctta atgcaacgtg gagagcgtgg

2281 atttagcggga acatcatccc ttgaagggtca atatggcatg atggcattcg atcttattta  
 2341 tcccgccaat cttgagcgtt ttgatcctaa tticactgcg aaaaagagtg tattagccgc  
 2401 tgataatcac ttaattttta ttggtagcaa tataaatagt agtgataaaa ataaaaatgt  
 2461 tgaacgacc ttattccaac atgccattac tccaacatta aatacccttt ggattaatgg  
 2521 aaaaaagata gaaaacatgc cttatcaaac aacacttcaa caagggtgatt ggtaattga  
 2581 tagcaatggc aatggttact taattactca agcagaaaaa gtaaatgtaa gtcgccaaca  
 2641 tcaggtttca gcggaaaata aaaatcgcca accgacagaa ggaaacttta gtcgggcatg  
 2701 gatcgatcac agcactcgcc ccaaagatgc cagttatgag tatatggctt ttttagatgc  
 2761 gacacctgaa aaaatgggag agatggcaca aaaattccgt gaaaataatg gggtatatca  
 2821 gggttctcgt aaggataaag acgttcatat tattctcgat aaactcagca atgtaacggg  
 2881 atatgccttt tatcagccag catcaattga agacaaatgg atcaaaaagg ttaataaacc  
 2941 tgcaattgtg atgactcatc gacaaaaaga cactcttatt gtcagtgcag ttacacctga  
 3001 tttaaatatg actcgccaaa aagcagcaac tcctgtcacc atcaatgtca cgattaatgg  
 3061 caaatggcaa tctgctgata aaaatagtga agtgaaatat caggtttctg gtgataacac  
 3121 tgaactgacg ttacgagtt actttgggtat tccacaagaa atcaaactct cgccactccc  
 3181 ttgatttaac caaagaacg ctcttgcgtt cctttttat ttgcaggaaa tctgattatg  
 3241 ctaataaaaa accctttagc ccacgcggtt acattaagcc tctgtttatc attaccgcga  
 3301 caagcattac ccactctgtc tcatgaagct ttcggcgata tttatcttt tgaagggtgaa  
 3361 ttaccaata cccttaccac ttcaataat aatcaattat cgctaagcaa acagcatgct  
 3421 aaagatgggtg aacaatcact caaatggcaa tatcaaccac aagcaacatt aacactaaat  
 3481 aatattgtta attaccaaga tgataaaaaat acagccacac cactcacttt tatgatgtgg  
 3541 atttataatg aaaaacctca atcttccca ttaacgttag catttaaaca aaataataaa  
 3601 attgactaa gttttaatgc tgaacttaac ttacggggtt ggcgaggtat tgctgttctt  
 3661 ttctgtgata tgcaaggctc tgcgacaggt caactgatc aattagtat caccgctcca  
 3721 aaccaagccg gaacactctt tttgatcaa atcatcatga gtgtaccgtt agacaatcgt  
 3781 tgggcagtac ctgactatca aacaccttac gtaaataacg cagtaaacac gatggttagt  
 3841 aaaaactgga gtgcattatt gatgtacgat cagatgttc aagcccatta cctacttta  
 3901 aacttcgata ctgaatttcg cgatgaccaa acagaaatgg cttcgattta tcagcgcttt  
 3961 gaatattatc aaggaattcc

**SEQ ID NO: 24** An HIV TAT sequence and Gly penta linker protein

G R K K R R Q R R R G G G G G

**SEQ ID NO: 25** Tat sequence and Gly penta linker nucleic acid

ggg cgt aaa aag cgt cgt caa cgt cgt cgt ggt ggt ggt ggt ggt

**SEQ ID NO: 26 Present Invention Chondroitinase ABC II Nucleic acid**

ttaccactctgtctcatgaagctttcggcgatatttatcttttgaaggcgaattaccaatataccttaccacttcaaataataatcaattatcg  
 ctaagcaaacagcatgctaaagatgggaacaatcactcaaatggcaatatcaaccacaagcaacattaacactaaataatattgttaatt  
 accaagatgataaaaatacagccacaccactcacttttatgatgtggatttataatgaaaacctcaatcttccccattaacgtagcattta  
 aaaaaataataaaattgcactaagttttaatgctgaacttaattttacggggtggcgaggtattgctgttcttttcgtgatatgcaaggctc  
 tgcgacaggtcaacttgatcaattagtatcaccgctccaaaccaagccggaacactctttttgatcaaatcatcatgagtgtagcattgta  
 gacaatcgttggcgagtacgtgactatcaaacaccttacgtaataacgcagtaaacacgatggtagtaaaaactggagtgcaattattg  
 atgtacgatcagatgtttcaagcccattaccctactttaaacctcgatactgaatttcgcgatgaccaaacagaaatggcttcgatttatcag  
 cgctttgaatattatcaaggaattcgtagtataaaaaattactccagatatgctagataaacatttagcggttatgggaaaaattgggggta  
 acacaacacgctgatggctcaatcacaggaaaagcccttgatcacccataaccggcaacattttatgaaagtcgaagggtgtatttagtga  
 ggggactcaaaaagcattacttgatgccaatatgctaagagatgtgggcaaacgcttctcaaactgctatttacttgcgtagcgattica  
 ttatcagcaactggtagaaaaaattagaagagcgctatttattaggtactcgttatgtccttgaacaagggttttacacgaggaagtgggtta

tcaaattattactcatgttggttaccaaacagagaacttttgcacatggtttattggccgcatgttcttgcaaaaaataaccttttagccc  
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actcaattgcaatggatgataaaaagcttattgatgtaccggattatcaacaacgtcaacaagccttagcgcgaactgcaaaagtggcta  
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cgccatttaaatggatggcattagcaggaacccagatggcaacaaaagttagataccacattatccgccgcttagcaaaacttagac  
aacaaaacgcattttgaaggcattaacgctgaaagttagccagtcggcgcatgggcaatgaattatgatcaatggcaatacaaacgaa  
gagcatcgaccaatcaccacaacaagctggctgccatagcgcgcgggttttagccgttatctgttggttaatgaaagctatgaaaaa  
acaaccgttatgctggttatttacaataatggacaattggaattattccagctgatttaactaatcagggttagccatgctggatgggatt  
ggaatagatatccaggtacaacaactatttcttccctataacgaactgaagcaaaaacttaatcaattacctgctgcaggtattgaaga  
aatgttgctttcaacagaaagtactctggtgcaaatacccttaataataacagtatgttgccatgaaattacacgggtcacagtaaatatca  
acaacaaagcttaagggcaataaatctatttctatttgataatagagttattgcttaggctcaggtattgaaatgatgataaacaacat  
acgaccgaaacaacactattccagtttgcctgcctaaattacagtcagtgatcattaatggcaaaaaggtaaataattagatactcaatt  
aactttaataatgcagatacattaattgatctgccggcaatttatataagctcactaaaggacaaactgtaaaattagttatcaaaaaca  
acattcacttgatgatagaaattcaaaaccaacagaacaattatttgcacagctgttatttctcatggttaaggcaccgagtaatgaaaat  
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ataaaataacccaagaagaggggatatgtgtttttgaagccactaagttaaaatcagcggatgcaacattattatccagtgatgcgccggt  
tatggtcatggctaaaaatacaaaatcagcaattaaacattaagttattgtaatcctgattaaatttatatcaaggtagagaaaaagatcaatt  
gatgataaaggtaatcaaatcgaagttagtggttattctctgcatggcttacagcagaatcgcaatcaacaaatagtactattaccgtaa  
aggaatatggaatfaacgacacctcaaccgggtgttattattaagcaccacaataacaacactcttattacgacaacaaccatacaggc  
aacacctactgttattaatttagttaagtaa

**SEQ ID NO: 27 Present Invention Chondroitinase ABC II protein**

```
>_ ABC (present invention) 990 aa vs.
>_ ABC (mature) 990 aa
scoring matrix: , gap penalties: -12/-2
98.3% identity; Global alignment score: 6393
```

	10	20	30	40	50	60
457676	LPTLSHEAFGDIYLFEGELPNILTTSSNNQLSLSKQHAKDGEQSLKWQYQPQATLTLNNI					
—	LPTLSHEAFGDIYLFEGELPNITLTTSSNNQLSLSKQHAKDGEQSLKWQYQPQATLTLNNI					
	10	20	30	40	50	60
	70	80	90	100	110	120
457676	VNYQDDKNTATPLTFMMWIYNEKPQSSPLTLAFKQNNKIALSFNAELNFTGWRGIAPVFR					
—	VNYQDDKNTATPLTFMMWIYNEKPQSSPLTLAFKQNNKIALSFNAELNFTGWRGIAPVFR					
	70	80	90	100	110	120
	130	140	150	160	170	180
457676	DMQGSATGQLDQLVITAPNQAGTLFFDQIIMSVPLDNRWAVPDYQTPYVNNVAVNTMVSKN					
—	DMQGSVTGQLDQLVITAPNQAGTLFFDQIIMSVPLDNRWAVPDYQTPYVNNVAVNTMVSKN					
	130	140	150	160	170	180
	190	200	210	220	230	240
457676	WSALLMYDQMFQAHYPTLNFDTEFRDDQTEMASITYQRFEYYQGIRSDKKITPDMLDKHLA					

	WSALLMYDQMFQAHYPTLNFDFE	RDDQTEMASRYQRF	EYYQGIRSDKKITPDMLDKHLA			
	190	200	210	220	230	240
	250	260	270	280	290	300
457676	LWEKLG	LTHADGSITGKALDHPNRQH	FMKVEGVFSEGTQKALLDANMLRDVGKTL	LQTA		
	.....	.....	.....	.....	.....	.....
	LWEKLV	LTHADGSITGKALDHPNRQH	FMKVEGVFSEGTQKALLDANMLRDVGKTL	LQTA		
	250	260	270	280	290	300
	310	320	330	340	350	360
457676	IYLRSD	SLSATGRKKLEERYLLGTRYVLEQGF	TRGSGYQIITHVGYQTRELFDAWFIGRH			
	.....	.....	.....	.....	.....	.....
	IYLRSD	SLSATDRKKLEERYLLGTRYVLEQGF	HRGSGYQIISHVGYQTRELFDAWFIGRH			
	310	320	330	340	350	360
	370	380	390	400	410	420
457676	VLAKNN	LAPTQQAMMWYNATGRIFEKDNEIVDANVDILNTQLQWMIKSL	LMLPDYQQRQ			
	.....	.....	.....	.....	.....	.....
	VLAKNN	LAPTQQAMMWYNATGRIFEKNNNEIVDANVDILNTQLQWMIKSL	LMLPDYQQRQ			
	370	380	390	400	410	420
	430	440	450	460	470	480
457676	QALAQ	LSWLNKTI	LSSKG	VAGGFKSDGSIFHHSQHYPAYAKDAFGGLAPSVYALSDSPF		
	.....	.....	.....	.....	.....	.....
	QALAQ	LSWLNKTI	LSSKG	VAGGFKSDGSIFHHSQHYPAYAKDAFGGLAPSVYALSDSPF		
	430	440	450	460	470	480
	490	500	510	520	530	540
457676	RLSTSA	HEHLKDVLLKMRIYTKETQIPV	VLSGRHPTGLHKIGIAPFKWMALAGTPDGKQK			
	.....	.....	.....	.....	.....	.....
	RLSTSA	HERLKDVLLKMRIYTKETQIPV	VLSGRHPTGLHKIGIAPFKWMALAGTPDGKQK			
	490	500	510	520	530	540
	550	560	570	580	590	600
457676	LDTTLS	AAAYANL	DNKTHFEGINAES	EPVGAWAMNYASMAIQRRASTQSPQQSWLAIARGF		
	.....	.....	.....	.....	.....	.....
	LDTTLS	AAAYAKL	DNKTHFEGIKAES	EPVGAWAMNYASMAIQRRASTQSPQQSWLAIARGF		
	550	560	570	580	590	600
	610	620	630	640	650	660
457676	SRYLVG	NESYENNNRYGRYLQYGQLEIIPADLTQSGF	SHAGWDWNRYPGTTT	TIHLPYNEL		
	.....	.....	.....	.....	.....	.....
	SRYLVG	NESYENNNRYGRYLQYGQLEIIPADLTQSGF	SHAGWDWNRYPGTTT	TIHLPYNEL		
	610	620	630	640	650	660
	670	680	690	700	710	720
457676	EAKLNQ	LPAAGIEEMLLSTESYSGANTLNNNSMFAMKLHG	HSKYQQQSLRANKSYFLFDN			
	.....	.....	.....	.....	.....	.....
	EAKLNQ	LPAAGIEEMLLSTESYSGANTLNNNSMFAMKLHG	PSKYQQQSLRANKSYFLFDN			
	670	680	690	700	710	720
	730	740	750	760	770	780
457676	RVIALG	SGIENDDKQHTTETTLFQFAVPKLQSVI	INGKKNQLD	TLTLNNADTLIDPAG		
	.....	.....	.....	.....	.....	.....
	RVIALG	SGIENDDKQHTTETTLFQFAVPKLQSVI	INGKKNQLD	TLTLNNADTLIDPAG		
	730	740	750	760	770	780
	790	800	810	820	830	840
457676	NLYKLT	KGQTVKFSYQKQHS	LDDRNSKPT	EQLFATAVISHGKAPS	NENYEYAI	IEAQNN
	.....	.....	.....	.....	.....	.....
	NLYKLT	KGQTVKFSYQKQHS	LDDRNSKPT	EQLFATAVISHGKAPS	NENYEYAI	IEAQNN



	790	800	810	820	830	840
	850	860	870	880	890	900
457676	KAPKYTVLQHNDQLHAVKDKITQEEGYGFFEATKLKSADATLLSSDAPVMVMAKIQNQQL					
	.....	.....	.....	.....	.....	.....
—	KAPEYTVLQHNDQLHAVKDKITQEEGYAFFEATKLKSADATLLSSDAPVMVMAKIQNQQL					
	850	860	870	880	890	900
	910	920	930	940	950	960
457676	TLNIVNPDNLNLYQGREKQFDDKGNQIEVSVYSRHWLTAESQSTNSTITVKGIWKLTPQ					
	.....	.....	.....	.....	.....	.....
—	TLNIVNPDNLNLYQGREKQFDDKGNQIEVSVYSRHWLTAESQSTNSTITVKGIWKLTPQ					
	910	920	930	940	950	960
	970	980	990			
457676	PGVLIKHHNNNTLITTTTIQATPTVINLVK					
	.....	.....	.....	.....	.....	.....
—	PGVLIKHHNNNTLITTTTIQATPTVINLVK					
	970	980	990			

# **SEQ ID NO: 28 Present Invention Chondroitinase ABC I nucleic acid**

gccaccagcaatcctgcattgacctaataatctgatgcagtcagaaattaccattttgcacaaaataaccattagcagacttctcatc  
 agataaaaactcaataactaacgttatctgataaacgtgacattatgggaaaccaatctctttatggaaatggaaagggtgtagtagcttta  
 cttacataaaaaactgattgtccccaccgataaagaagcatctaaagcatggggacgctcatccacccccgtttctcattttggctttac  
 aatgaaaaaccgattgatggtatcttactatcgtttcggagaaaaactcattcaaccagtgaggctcaggcaggctttaaagttaaatt  
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 atggtactcaagacagcattggcggttcttttaggtgctaaagtcgatagtattcgttttaaagcgcttctaatgtgagtcagggtgaaatc  
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 gtgaaaaagagacaaacctgcattagaagagaatatcagcaaatfaaaagtgtttcgatgctcttaatactcacactttagcaaatg  
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 aagcagatgtacttattaatgacaaagcatttattagatcaaggcttgttaaggagggtgcttttagtgacnaccatcactggggataca  
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 tcacgtgagtttaaaagtagttttgatatgaaagtaagtgtgatagctctgatctagattattcaataccttatctcgccaacatttagcctt  
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 gtggttaaagatggtttacgccctgatggtacagcatggcgacatgaaggcaactatccgggctactctttccagccttfaaaatgcct  
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 acagtaatccagaagtggattaccgcttcaggaagacacctcttaactcaccitcgttaaaatcagtcgctcaaggcttattactggct  
 tgccatgtctgcaaaatcgcctgataaaacacttgcatttattatcttgcgattagtataaaacacaaaatgaatcaactgctatttt  
 ggagaaactattacaccagcgtctttacctcaaggtttctatgcctttaatggcggtgcttttggtattcgttggtgcaagataaaatggtg  
 aactgaaagcttataacaccaatgtttggtcatctgaaatttataacaaagataaccgttatggccgttaccaaagtcatggtgtcgtca  
 aatagtgtatgtgctgcagctttcacagggtatcagcaagaagggtgggattggaatagaatgccaggggcaaccactatccac  
 ctctcttaaaagacttagacagtcctaaacctcataccttaatgcaacgtggagagcgtggatttagcggaacatcatcccttgaaggctc  
 aatatggcatgatggcattcgtcttattatcccgccaatcttgagcgtttgatcctaatttactgcgaaaaagagtgtattagccgctg  
 ataactacttaattttattgtagcaataataatagtagtgataaaaataaaaatgttgaaacgaccttattccaacatgccattactccaac  
 attaaataccttttgattaatggacaaaagatagaaaacatgccttatcaaaacacttcaacaagggtgattggttaattgatagcaatg  
 gcaatggttacttaattactcaagcagaaaaagttaagtgaagtcgccaacatcaggtttcagcggaataaaaaatcgccaaccgaca  
 gaaggaaactttagctcggcatggatgatcacgacactgccccaaagatgccagttatgagtatggcttttttagatgcgacacct  
 gaaaaaatgggagagatggcacaataatccgtgaaaataatgggttatatcaggttcttgtaaggataaagacgttcatatttctc  
 ataaactcagcaatgtaacgggatgacctttatcagccagcatcaattgaagacaaatggatcaaaaagggttaataaacctgcaattgt  
 gatgactcatcgacaaaaagacactcttattgtcagtcagttacacctgatttaaatatgactcgccaaaaagcagcaactcctgtcacc

atcaatgtcacgattaatggcaaatggcaatctgctgataaaaatagtgaagtgaatatcaggtttctggtgataacactgaactgacgt  
ttacgagttactttggtattccacaagaatacaactctcgccactccctga

# **SEQ ID NO: 29 Present Invention Chondroitinase ABC I protein**

365019 ATSNPAFDPKNLMQSEIYHFAQNNPLADFSSDKNSILTLSDKRSIMGNQSLWKKWKGSS

.....

365019 FTLHKKLIVPTDKEASKAWGRSSTPVFSFWLYNEKPIDGYLTIDFGEKLISTSEAQAGFK

365019 VKLDFGTWRTVGVSLNNDLENREMTLNATNTSSDGTQDSIGRSLGAKVDSIRFKAPSNVS

365019 QGEIYIDRIMFSVDDARYQWSYQVKTRLSEPEIQFHNVPQLPVT PENLAAIDLIRQRL

365019 INEFVGGEKETNLALEENISKLSDFDALNTHTLANGGTQGRHLITDKQIIYQPENLNS

365019 QDKQLFDNYVILGNYTTLMFNISRAYVLEKDPQKAQLKQMYLLMTKHLLDQGFVKGSAL

365019 VTTHHWGYSSRWYISTLLMSDALKEANLQTQVYDSSLWYSREFKSSFDMKVSADSSDL

365019 YFNTLSRQHLALLLEPDDQKRINLVNTFSHYITGALTQVPPGGKDGLRPDGTAWRHEGN

365019 YPGYSFPAFKNASQLIYLLRDTFVSVGESGWNLSKKAMVSAWIYSNPEVGLPLAGRHLN

365019 SPSLKSVAQGYWLAMSAKSSPDKTLASIYLAISDKTQNESTAIFGETITPASLPQGFYA

365019 FNGGAFGIHRWQDKMVTLKAYNTNVWSSEIYNKDNRYGRYQSHGVAQIVSNGSQLSQGYQ

365019 QEGWDWNRMPGATTIHLPLKDLDSPKPHTLMQRGERGFSGTSSLEGQYGMMAFDLIYPAN

365019 LERFDPNFTAKSVLAADNHLIFIGSNINSSDKNKNVETTLFQHAIPTLNTLWINGQKI

365019 ENMPYQTTLQQGDWLIDSNGNGYLITQAEKVNVSQRHQVSAENKNRQPTEGNFSSAWIDH

365019 STRPKDASYEYMVFLDATPEKMGEMAKQFRENGLYQVLRKDKDVHIILDKLSNVTGYAF

365019 YQPASIEDKWKVKNKPAIVMTHRQKDTLIVSAVTPDLNMTRQKAATPVTINVTINGKWQ

365019 SADKNSEVKYQVSGDNTELTFTSYFGIPQEIKLSPLP

hyaluronidase 1 protein, **SEQ ID NO: 30**, Locus HSU96078

hyaluronidase 2 protein, **SEQ ID NO: 31**,

hyaluronidase 3 protein, **SEQ ID NO: 32**, Locus BC012892

hyaluronidase 4 protein, **SEQ ID NO: 33**, AF009010

PH-20 protein, **SEQ ID NO: 34**,

Tat peptide **SEQ ID NO: 35**

ABCI a site-specific mutant designated H501a and Y508a **SEQ ID NO: 36**

**SEQ ID NO: 37** Chondroitinase ABCI protein Locus P59807

## **ORIGIN**

1 mpifrtala mtlgllsapy namaatsnpa fdpknlmqse iyhfaqnnpl adfssdknsi  
61 ltlsdkrsim gnqslwkwk ggssftlhkk livptdkeas kawgrsstp v fswlynekp  
121 idgyltidfg eklistsea agfkvkl dft gwrvagvsl n ndlenremtl natntssdgt  
181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqv k trlsepeiqf

241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan  
 301 ggtqgrhlit dkqiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdpqtka  
 361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvysd  
 421 llwysrefks sfdmkvsads sdldyfntls rqlalalle pddqkrinlv ntfshtyitga  
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk  
 541 amvsawiyysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk  
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr  
 661 ygryqshgva qivsngsqsls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger  
 721 fgsgtssleg qygmmafni ypanlerfdp nftakksvla adnhlfigs ninssdknkn  
 781 vetltfghai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq  
 841 hqvsaenknr qptegnfsa widhstrpkd asyeymvfld atpekmngema qkfrenngly  
 901 qvlrkdvdh iildklsnvt gyafyqpasi edkwikkvkn paivmthrqr dtlivsavgp  
 961 dlnmtrqkaa tptinvtn gkwqsadkns evkyqvsgdn telftsytfg ipqeiklspl  
 1021 pXX

**SEQ ID NO: 38** NA20 ABCI (A<sub>45</sub>-N<sub>1023</sub>), protein

aqnnpl adfssdknsi

61 ltlsdkrsim gnqslwkwwk ggssftlhkk livptdkeas kawgrsstpvsfswlynekp  
 121 idgyltidfg eklistseaq agfkvkldft gwravgvsln ndlenremtl natntssdgt  
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf  
 241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan  
 301 ggtqgrhlit dkqiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdpqtka  
 361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvysd  
 421 llwysrefks sfdmkvsads sdldyfntls rqlalalle pddqkrinlv ntfshtyitga  
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk  
 541 amvsawiyysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk  
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr  
 661 ygryqshgva qivsngsqsls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger  
 721 fgsgtssleg qygmmafni ypanlerfdp nftakksvla adnhlfigs ninssdknkn  
 781 vetltfghai tptlntlwin gqkienmpyq ttlqqgdwli dsngngylit qaekvnvsrq  
 841 hqvsaenknr qptegnfsa widhstrpkd asyeymvfld atpekmngema qkfrenngly  
 901 qvlrkdvdh iildklsnvt gyafyqpasi edkwikkvkn paivmthrqr dtlivsavgp  
 961 dlnmtrqkaa tptinvtn gkwqsadkns evkyqvsgdn telftsytfg ipqeiklspl  
 1021 pXX

**SEQ ID NO: 39** NA60 ABCI (F<sub>85</sub>-N<sub>1023</sub>) protein

ftlhkk livptdkeas kawgrsstpvsfswlynekp

121 idgyltidfg eklistseaq agfkvkldft gwravgvsln ndlenremtl natntssdgt  
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf  
 241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan  
 301 ggtqgrhlit dkqiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdpqtka  
 361 qlkqmyllmt khlldqgfvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvysd  
 421 llwysrefks sfdmkvsads sdldyfntls rqlalalle pddqkrinlv ntfshtyitga  
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk  
 541 amvsawiyysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk  
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr

661 ygryqshgva qivsngsqsls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger  
 721 gfgstssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn  
 781 vettlfqhai tptlntlwin gqkienmpyq tllqqgdwli dsngngylit qaekvnvsrq  
 841 hqvsakenr qptegnfsa widhstrpkd asyeymvfld atpekmgema qkfrenngly  
 901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlvsavtp  
 961 dlnmtrqkaa tptvntvin gkwqsadkns evkyqvsgdn telftsifg ipqeiklspl  
 1021 pXX

**SEQ ID NO. 40:** NΔ60 CΔ80 ABCI (F<sub>85</sub>-A<sub>942</sub>) protein

ftlhkk livptdkeas kawgrsstpvs fswlynekp  
 121 idgyltidfg eklistseag agfkvkl dft gwravgvsl nndlenremtl natntssdgt  
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepei qf  
 241 hnvkpqlpvt penlaaidli rqrlinefv geketnlale enisklksdf dalnihtlan  
 301 ggtqgrhlit dkqiiyqpe nlmsqdkqlf dnyvilgnyt tlmfnisray vlekdptqka  
 361 qlkqmyllmt khldqgfvk gsalvtthw gyssrwwyis tlmsdalke anlqtqvysd  
 421 llwysrefks sfdmkvsads sldyfntls rqlhlalle pddqkrinlv ntfshyitga  
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk  
 541 amvsawiy sn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk  
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr  
 661 ygryqshgva qivsngsqsls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger  
 721 gfgstssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn  
 781 vettlfqhai tptlntlwin gqkienmpyq tllqqgdwli dsngngylit qaekvnvsrq  
 841 hqvsakenr qptegnfsa widhstrpkd asyeymvfld atpekmgema qkfrenngly  
 901 qvlrkdkdvh iildklsnvt gyafyqpasi edkwikkvnk pa

**SEQ ID NO. 41:** I. Nucleotide sequence for TAT-ABCI-nΔ20:

1 ggtc gtaaaaagcg tcgtcaacgt cgtcgtcctc ctcaatgcgc acaaaataac  
 61 ccattagcag acttctcacc agataaaaac tcaataactaa cgttatctga taaacgtacg  
 121 attatgggaa accaatctct tttatggaaa tggaaagggtg gtagtagctt tactttacat  
 181 aaaaaactga ttgtcccccac cgataaagaa gcatctaaag catggggacg ctcaccacc  
 241 cccgttttct cattttggct ttacaatgaa aaaccgattg atggttatct tactatcgat  
 301 ttcggagaaa aactcatttc aaccagttag gctcaggcag gctttaaagt aaaattagat  
 361 ttcactggct ggcgtactgt gggagtctct ttaaataacg atcttgaaaa tcgagagatg  
 421 accttaaatg caaccaatac ctctcttgat ggtactcaag acagcattgg gcgttcttta  
 481 ggtgctaaag tcgatagtat tcgttttaaa gcgccttcta atgtgagtca gggtgaaatc  
 541 tatatcgacc gtattatggt ttctgtcgat gatgctcgct accaatgggtc tgattatcaa  
 601 gtaaaaactc gcttatcaga acctgaaatt caatttcaca acgtaaagcc acaactacct  
 661 gtaacacctg aaaatttagc ggccattgat cttatcgcc aacgtctaataatgaattt  
 721 gtcggagggtg aaaaagagac aaacctcgca ttagaagaga atacagcaa attaaaaagt  
 781 gatttcgatg ctcttaatac tcacacttta gcaaatgggtg gaacgcaagg cagacatctg  
 841 atcactgata aacaaatcat ttttatcaa ccagagaatc ttaactctca agataaaca  
 901 ctatttgata attatggtat tttaggtaat tacacgacat taatgtttta tattagccgt  
 961 gcttatgtgc tggaaaaaga tcccacacaa aaggcgcaac taaagcagat gtacttatta  
 1021 atgacaaagc atttattaga tcaaggcttt gttaaaggga gtgctttagt gacnaccat  
 1081 cactggggat acagttctcg ttggtggtat attccacgt tattaatgtc tgatgcacta  
 1141 aaagaagcga acctacaaac tcaagtttat gattcattac tgtgggtatc acgtgagttt  
 1201 aaaagtagtt ttgatatgaa agtaagtgtc gatagctctg atctagatta tttcaatacc

1261 ttatctgcc aacatttagc cttattacta ctagagcctg atgatcaaaa gcgtatcaac  
1321 ttagttaata ctttcagcca ttatatcact ggcgcatata cgcaagtgcc accgggtggt  
1381 aaagatggtt tacgccctga tggtagacga tggcgacatg aaggcaacta tccgggctac  
1441 tctttccag ccttaaaaa tgcctctcag cttatttatt tattacgca tacaccattt  
1501 tcagtgggtg aaagtgggtg gaatagcctg aaaaaagcga tggtttcagc gtggatctac  
1561 agtaatccag aagttggatt accgcttgca ggaagacacc ctcttaactc accttcgta  
1621 aaatcagtcg ctcaaggcta ttactggctt gccatgtctg caaatcatc gcctgataaa  
1681 aacttgcat ctatttatct tgcgattagt gataaacac aaaatgaac aactgctatt  
1741 ttggagaaa ctattacacc agcgtctta cctcaagggt tctatgcctt taatggcgtt  
1801 gcttttgga ttcacgttg gcaagataaa atggtgacac tgaaagctta taacaccaat  
1861 gtttggtcat ctgaaattta taacaaagat aaccgttatg gccgttacca aagtcatggt  
1921 gtcgctcaaa tagtgagtaa tggctcgag ctttcacagg gctatcagca agaagggttg  
1981 gattggaata gaatgccagg ggcaaccact atccacctc ctcttaaga cttagacagt  
2041 cctaaacctc ataccttaat gcaacgtgga gagcgtggat ttagcggaa atcatccctt  
2101 gaaggtcaat atggcatgat ggcattcgat cttattatc cggccaatct tgagcggtt  
2161 gatcctaatt tctcgcgaa aaagagtga ttagccgctg ataactactt aattttatt  
2221 gtagcaata taaatagtag tgataaaaa aaaatgttg aaacgacctt attccaacat  
2281 gccattactc caacattaaa tacccttggg attaatggac aaaagataga aaacatgcct  
2341 tatcaacaaa cactcaaca aggtgattgg ttaattgata gcaatggcaa tggttactta  
2401 attactcaag cagaaaaagt aaatgtaagt cgccaacatc aggtttcagc ggaaaaataa  
2461 aatcgccaac cgacagaagg aaacttagc tcggcatgga tcgatcacag cactcgcccc  
2521 aaagatgcca gttatagta tatggtctt ttagatgca cacctgaaaa aatgggagag  
2581 atggcaciaa aattccgtga aaataatggg ttatatcagg ttctcgtaa ggataaagac  
2641 gtcatatta ttctgataa actcagcaat gtaacgggat atgccttta tcagccagca  
2701 tcaattgaag acaaatggat caaaaagggt aataaacctg caattgtgat gactcatga  
2761 caaaaagaca ctctattgt cagtgcagt acacctgatt taaatatgac tcgcaaaaa  
2821 gcagcaactc ctgtcaccat caatgtcacg attaatggca aatggcaatc tgctgataa  
2881 aatagtgaag tgaaatatca ggttctggt gataaactg aactgacgtt tacgagttac  
2941 ttggtattc cacaagaaat caactctcg ccactccctt ga

**SEQ ID NO. 42: II. Amino acid sequence for TAT-ABCI-nΔ20:**

grkkrqrppqcaqnnpladfssdknsiitlsdkrsimgnqslwkwkgssftlhkkli vptdkeaskawgrsstpvfsfw  
lynekipidgyltidfgeklstseaqagfkvkl dftgwrvtgvslnndlenremtlnatntssdgtqdsigrslgkavdsirfkapsnv  
sqgeiyidrimfsvddaryqwsdyqvtrlsepeiqfhnvkpqlpvtpenlaaidlirqlinefvggeketnlaleenisklsdfe  
alnhtlanggtqgrhlitdkqiiyqpenlnsqdkqlfdnyvilgnyttlmfnisrayvlekdptqkaqlkqmyllmtkhllldqgfv  
kgsalvtthhwgyssrwwyistilmsdalkeanlqtqvysllwysrefkssfdmkvsadssldyfnltlrqlhlalllepddqkr  
inlvntfshyitgaltqvppggkdglrpdgtawrhegnypgysfpafknasqliyllrdtpfsvgesgwslkkamvsawiysnp  
evglplagrplnspslksvaqgywlamsspdktlasiylaisdktqnestaifgetitpaslpqgfya fnggafgihrwqdk  
mvtlkayntnvwssseynkdnrygryqshgvaqivnsqslsqgyqqegwdwnrmpgattihlplkdlspkphltmqrger  
gfsgtsslegqygmmafdiipyanlerfdpnftakksvlaadnhlifgsninsdknkvettlfqhaitptlntwingqkienm  
pyqttlqqgdwlidsngngylitqaekvnvsrqhqvsaenknrqptegnssawidhstrpkdas yeymvfl datpekm gema  
qkfrennglyqvlrkdvdhiiidklsnvtgyafyqpasiedkwikkvnpaivmthrqkdtlivs avtpdlnmtrqkaatpvtin  
vtingkwqsadknsevkyqvs gdneltfts yfgipqeikslplp

**SEQ ID NO. 43: II. Nucleotide sequence for TAT-ABCI-nΔ60:**

ggctgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctttactttacataaaaaactgattgtccccaccgataaagaagcatcta  
aagcatggggacgtcatccacccccgttttctcatttggctttacaatgaaaaaccgattgatggttatcttactatcgatttcggagaaa  
aactcatttcaaccagtgaggctcaggcaggctttaaagtaaaattagatttactggctggcgtactgtgggagctctttaaataacgat  
cttgaatacgtagagatgaccttaaatgcaaccaatacctcctctgatggactcaagacagcattggcggttcttaggtgctaaagtcg  
atagtattcgttttaaacgccttctaattgtgagtcagggtgaaatctatcgcaccgtattatgttttctgctgatgatgctcgtaccaatg  
gtctgattatcaagtaaaaactgccttatcagaacctgaaattcaatttcacaacgtaaagccacaactacctgtaacacctgaaaatttag  
cggccattgatcttattcgtcaacgtctaatgaatttgcggaggtgaaaaagagacaaacctgcattagaagagaatatcagca  
aattaaaaagtgatttcgatgcttctaatactcacactttagcaaatgggtggaacgcaaggcagacatctgatcactgataaacaatcatt  
atttatcaaccagagaatcttaactctcaagataaacaactatttgataattatgtatttttagtaattacacgacattaatgtttaatttagc  
cgtgcttatgtgctggaaaaagatccacacaaaaggcgcaactaaagcagatgtacttattaatgacaaagcatttattagatcaaggc  
tttgtaaaagggagtgcttttagtgacnaccatcactggggatagcagttctcgttggtggtatattccacgttattaatgtctgatgactaa  
aagaagcgaacctacaaactcaagtttatgattcattactgttggtattcacgtgagtttaaaagtagtttgatatgaaagtaagtgctgata  
gctctgatctagattatttcaataccttatctgccaacatttagccttattactactagagcctgatgatcaaaagcgtatcaacttagttaat  
actttcagccattatcactggcgcatcgaagtgccaccgggtggttaaagatgggttacgccctgatggtacagcatggcgacat  
gaaggcaactatccgggctactctttccagcctttaaataatgcctctcagcttatttatttacgcgatacaccatttcagtggtgaa  
agtggttggaatagcctgaaaaaagcagtggtttcagcgtggatctacagtaatccagaagttggattaccgcttcaggaagacacc  
tcttaactcacctcgttaaaatcagtcgctcaaggctattactggcttgccatgtctgcaaaatcatcgctgataaaacacttgcatttatt  
tatcttgcgatttagtataaaacacaaaatgaatcaactgctatttttgagaaaactattacaccagcgtctttacctcaaggtttctatgcct  
ttaatggcgggtgcttttggtattcatcgttggaagataaaatggtgacactgaaagcttataacaccaatgtttggtcatctgaaattataa  
caaagataaccgttatggcgttaccaaagtcaggtgtcgtcaaatagtgagtaatggctcgcagctttcacagggtatcagcaag  
aaggttgggattggaatagaatgccaggggcaaccactatccaccttctttaaagacttagacagtcctaaacctataccttaatgc  
aacgtggagagcgtgatttagcgaacatcatccctgaaggtcaatatggcatgatggcattcgtatcttattatcccgccaatcttga  
gcgtttgatcctaatttactgcgaaaaagagtgatttagccgctgataatcacttaattttattggtagcaatataaataagtagtgataaa  
aataaaaatgftgaacgaccttattccaacatgccattactccaacattaataaccctttgattaatggacaaaagatagaaaacatgc  
cttatcaacaacacttcaacaagggtgattggttaattgatagcaatggcaatggttacttaattactcaagcagaaaaagtaaatgtaagt  
cgccaacatcaggtttcagcggaaaaataaaaatcgccaaccgacagaaggaaacttttagctcggcatggatcgatcacagcactcgc  
cccaaagatgccagttatgagtatatggtcttttagatgcgacacctgaaaaaatgggagagatggcacaaaaattccgtgaaaaaat  
gggttatatcaggtttctgtaaggataaagacgttcattattctcgataaactcagcaatgtaacgggatatgccttttatcagccagca  
tcaattgaagacaaatgatcaaaaagggttaataaacctgcaattgtgatgactcatcgacaaaaagacacttattgtcagtcaggtta  
cacctgatttaaatatgactcgcaaaaagcagcaactcctgtccatcaatgtcacgattaatggcaaatggcaatctgtgataaaa  
atagtgaaagtgaatatcaggtttctggtgataacactgaactgacgtttacgagttactttggtattccacaagaatacaactctcgcca  
ctccctga

#### SEQ ID NO. 44: IV. Amino acid sequence for TAT-ABCI-nΔ60

grkrrrrppqcfllhkkliivptdkeaskawgrsstpvfswlynekipidgyltidfgeklisetaaqgfkvklfdtgwrtvgvsl  
nndlenremtlnatntssdgtqdsigrslgakvdsirfkapsnsvsqgeiyidrimfsvddaryqwsdyqvktlrlsepeiqfhnvkv  
qlpvtpenlaaidlirqlinefvggeketnlaleeniskksdfdalnthlanggtqgrhlitdkqiiiyqpenlnsqdkqlfdnyvil  
gnytlmfnisrayvlekdptqkaqlkmyllmtkhlldqgfvkgsalvtthhwgyssrwwyistllmsdalkeanlqtqvysdl  
wysrefkssfdmkvsadssldyfnlrsqhlallllepddqkrinlvntfshyitgaltqvppggkdglrpdgtawrhegnypgys  
fpafknasqliyllrdtpfsvgesgwnslkkamvsawiysnpevgplagrhplnspslksvaqgyywlamsaksspdktlasiy  
laisdktqnestaifgetitpaslpqgfayfnggafgihrwqdkmvtlkayntnvwssieynkdnrygryqshgvaqivsnsgqls  
qgyqqegwdwnrmpgattihlplkldspkphltlmqrgergfsstsgqygmmafddiypanlerfdpnftakksvlaadn  
hlifignsinssdknkvettlfqhaitptlntwingqkienmpyqtlqqgdwldsnngnylitqaekvnvsrqhqvsakenr  
qptegnfsawidhstrpkdaseymvfldatpekmngemaqkfrennglyqvlrkdvdvhiildklsnvtgyafyqpasiedk  
wikknkpaivmthrqkdtilvsavtpdlmtrqkaatpvtinvtngkwqsadknsevkyqvsdnteltfysyfgipqeiklsp  
lp

**SEQ ID NO. 45: V. Nucleotide sequence for ABCI-TAT-C:**

gccaccagcaatcctgcatttgatcctaaaaatctgatgcagtcagaaattaccatttgcacaaaataacccattagcagacttctcatc  
agataaaaactcaactaactacgttatctgataaacgtagcattatgggaaaccaatctctttatggaaatggaaagggtgtagtagcttta  
ctttacataaaaaactgattgtccccaccgataaagaagcatctaaagcatggggacgctcatccacccccgtttctcattttggctttac  
aatgaaaaaccgattgatgttatcttactatcgatttcggagaaaaactcatttcaaccagtgaggctcaggcaggctttaagtaaaatt  
agatttactggctggcgtactgtgggagctctttaataaacgatcttgaaaatcgagagatgaccttaaatgcaaccaatactcctctg  
atgggtactcaagacagcattgggcgttcttaggtgctaaagtcgatagtattcgtttaaagcgccttctaattgtgagtcagggtgaaatc  
tatacgcacgtattatgtttctgtcgtatgctcgtaccaatggctgattatcaagtaaaaactcgttatcagaacctgaaattcaatt  
tcacaacgtaaaagccacaactacctgtaaacctgaaaatttagcggccattgatcttattcgccaacgtctaattaatgaattgtcggag  
gtgaaaaagagacaaacctcgcattagaagagaatatcagcaaaftaaaaagtatttcgatgctcttaatactcacactttagcaaatg  
gtggaacgcaaggcagacatctgatcactgataaacaatcattattatcaaccagagaatcttaactctcaagataaacaactatttgat  
aattatgtatttttagtaattacacgacattaatgtttaatattagccgtgcttatgtgctggaagagatccacacaaaaggcgaacta  
aagcagatgtacttattaatgacaaagcattattagatcaaggccttgttaaaggagtgcttttagtgacnaccatcactggggataca  
gttctcgttggtgttatattccacgttattaatgtctgatgcactaaaagaagcgaacctacaaactcaagtttatgattcattactgtggtat  
tcacgtgagtttaaaagtagtttgatagaaagtaagtctgatagctctgatctagattatttcaataccttatctcgccaacatttagcctt  
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gtggtaaagatggttacgccctgatggtacagcatggcgacatgaaggcaactatccgggctactctttccagcctttaaaaatgcct  
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aatagtgagtaatggctcgcagcttcacagggctatcagcaagaaggttgggattggaatagaatgccaggggcaaccactatccac  
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aatatggcatgatggcattcgtatcttattatcccgccaatcttgagcgttttgatcctaatttactgcgaaaaagagtgtattagccgctg  
ataatcacttaattttattgtagcaatataaatagtagtataaaaaataaaatgttgaacgacctattccaacatgccattactccaac  
attaaatacccttggattaatggacaaaaagatagaaaacatgccttatcaacaacacttcaacaaggtgattggttaattgatagcaatg  
gcaatggttacttaattactcaagcagaaaaagttaagtgaagtcgccaacatcaggtttcagcggaaaaataaaaatcgccaaccgaca  
gaaggaaacttagctcggcatggtatgcacagcactcgcaccaagatgccagttatgagtatatggtcttttagatgcgacacct  
gaaaaaatgggagagatggcacaaaaattccgtgaaaataatgggttatatcaggttctcgttaaggataaaagacgttcatattattctc  
ataaactcagcaatgtaacgggatatgcctttatcagccagcatcaattgaagacaaatggatcaaaaaggttaataaacctgcaattgt  
gatgactcatcgacaaaaagacactcttattgtcagtgagttacacctgatttaaatgactcgccaaaaagcagcaactcctgtcacc  
atcaatgtcacgattaatggcaatggcaatctgctgataaaaatagtgaagtgaatatcaggtttctggtgataaactgaactgacgt  
ttacgagttactttggtattccacaagaaatcaactctcgccactccct  
ggctgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctag

**SEQ ID NO. 46: V. Amino acid sequence for ABCI-TAT-C:**

atsnpafdpknlmqsei yhfaqqnnpladfssdknsiltlsdkrsimgnqsl lkwkkgssftlhkkli vptdkeaskawgrsstpv  
fsfwlynekpidgyltidfgeklistseaqagfkvkl dftgwrtvgvsl nndlenremtlnatntssdgtqdsigrslgakvdsirfka  
psnvsqgeiyidrimfsvddaryqwsdyqvktlrsepeiqfhvnpqlpvtpenlaaidlirqlinefvggeketnlaleenisklk  
sdfdalnthl langgtqgrhlitdkqii yqpenlnsqdkqlfdnyvilgnyttlmfnisrã yvlekdp tqkaqlkqmyllmtkhll d  
qgfvkgsalvtthhwgyssrwwyistilmsdalkeanlqtqvysdl l wysrefkssfdmkvsadssldlyfntlsrqlhal llllep d  
dqkrinlvntfshyitgaltqvp ppgkdglrpdgtawrhegnypgysfpafknasqli yllrdtpfsvgesgwnslkkamvsawi  
ysnpevglplagrhlpspslksvaqgyywlamsaksspdktlasiylaisdktnestafgetitpaslpqgfya fnggafgihr  
wqdkmvtlkayntnvwsssei ynkdnrygryqshgvaqivsnsgslsqgyqqegwdwnrmpgattihlpkldldspkphltm  
qrgergfsstsslegqygmmafdli ypanlerfdpnftakksvlaadnhlifigninssdknknvettl fqhaitptlntl wingqki



enmpyqtlqqgdwlidsngngylitqaekvnvsrqhqvsakenrqpTEgnfssawidhstrpkdasYeymvflDatpekmgemaqkfrennglyqvlrkdKdvhiildklsnvtgyafyqpasiedkwikkvnkpaivmthrQkdtlivsavtpdlnmtrqkaa  
tpvtinvtingkwqsadknsevkyqvsGdnteltfysyfgipqeiklsplpgrkkrrrrppqc

SEQ ID NO. 47 Nucleotide sequence for chondroitinase ABCI-nΔ20

gc acaaaataac  
61 ccattagcag acttctcatc agataaaaac tcaataactaa cgttatctga taaacgtagc  
121 attatgggaa accaatctct tttatggaaa tggaaagggtg gtagtagctt tactttacat  
181 aaaaaactga ttgtcccccac cgataaagaa gcatctaaag catggggacg ctcattccacc  
241 cccgttttct cattttggct ttacaatgaa aaaccgattg atggttatct tactatcgat  
301 ttcgagagaaa aactcatttc aaccagtgag gctcaggcag gctttaaagt aaaattagat  
361 ttactggct ggcgtactgt gggagtctct taaataacg atcttgaata tcgagagatg  
421 accttaaatg caaccaatac ctctctgat ggtactcaag acagcattgg gcgttctta  
481 ggtgctaag tcgatagtat tcttttaaa gcgccttcta atgtgagta gggtgaaatc  
541 tatatcgacc gtattatgtt ttctgtgat gatgctcgt accaatggtc tgattatcaa  
601 gtaaaaactc gcttatcaga acctgaaatt caattcaca acgtaaagcc acaactacct  
661 gtaacacctg aaaatttagc ggccattgat cttattcgc aacgtctaataa taatgaatt  
721 gtcggagggtg aaaaagagac aaacctgca ttagaagaga atatcagcaa attaaaaagt  
781 gatttcgatg ctcttaatac tcacacttta gcaaatgggtg gaacgcaagg cagacatctg  
841 atcactgata acaaatcat tatttatcaa ccagagaatc ttaactctca agataaaca  
901 ctatttgata attatgttat ttaggtaata tacacgacat taatgtttaa tattagccgt  
961 gcttatgtgc tggaaaaaga tcccacacaa aaggcgcaac taaagcagat gtacttatta  
1021 atgacaaagc atttattaga tcaaggcttt gttaaaggga gtgctttagt gacnaccat  
1081 cactggggat acagtctcg ttgggtggtat atttcacgt tattaatgtc tgatgcacta  
1141 aaagaagcga acctacaaac tcaagtttat gattcattac tgtggtattc acgtgagttt  
1201 aaaagtagtt ttgatatgaa agtaagtgtc gatagctctg atctagatta tttaatacc  
1261 ttatctcgcc aacatttagc cttattacta ctagagcctg atgatcaaaa gcgtatcaac  
1321 ttagttaata ctttcagcca ttatatact ggcgcattaa cgcaagtgcc accgggtggt  
1381 aaagatggtt tacgccctga tggtagcga tggcgacatg aaggcaacta tccgggctac  
1441 tcttccag ccttataaaa tgctctcag cttattatt tattacgga tacaccatt  
1501 tcagtgggtg aaagtgggtg gaatagcctg aaaaagcga tggttcagc gtgatctac  
1561 agtaatccag aagtggatt accgcttga ggaagacacc ctcttaactc accttcgtta  
1621 aatcagtcg ctcaaggcta ttactggctt gccatgtctg caaatcatc gcctgataaa  
1681 aacttgcat ctattatct tgcgattagt gataaaacac aaaatgaatc aactgctatt  
1741 ttggagaaa ctattacacc agcgtctta cctcaaggtt tctatgcctt taatggcggt  
1801 gcttttgta ttcacgttg gcaagataaa atggtgacac tgaagctta taacaccaat  
1861 gtttggtcat ctgaaattta taacaaagat aaccgttatg gccgtacca aagtcaggt  
1921 gtcgtcaaaa tagtgagtaa tggctgcag cttcacagg gctatcagca agaaggttg  
1981 gattggaata gaatgccagg ggcaaccact atccacctc ctctaaaga ctagacagt  
2041 cctaaacctc ataccttaat gcaacgtgga gagcgtggat ttagcggaac atcatccct  
2101 gaaggtcaat atggcatgat ggcattcgat cttattatc ccgccaatct tgagcgttt  
2161 gacctaatt tactgcgaa aaagagtga ttagccgtg ataactactt aattttatt  
2221 ggtagcaata taaatagtag tgataaaaat aaaaatgtg aaacgacctt attccaacat  
2281 gccattactc caacattaaa tacctttgg attaatggac aaaagataga aaacatgcct  
2341 tatcaaaaa cactcaaca aggtgattgg ttaattgata gcaatggcaa tggttactta  
2401 attactcaag cagaaaaagt aatgtaagt cgccaacatc aggttcagc ggaaaaataa  
2461 aatcgccaac cgacagaagg aaacttagc tcggcatgga tcgatcacag cactcgcccc  
2521 aaagatgcca gttatagta tatggtctt ttagatgca cacctgaaaa aatgggagag  
2581 atggcacaaa aattccgtga aaataatgg ttatatcagg ttctcgtaa ggataaagac



2641 gtccatatta ttctcgataa actcagcaat gtaacgggat atgcctttta tcagccagca  
2701 tcaattgaag acaaatggat caaaaagggt aataaacctg caattgtgat gactcatcga  
2761 caaaaagaca ctcttattgt cagtgcagtt acacctgatt taaatatgac tcgccaaaaa  
2821 gcagcaactc ctgtcacat caatgtcacg attaatggca aatggcaatc tgctgataaa  
2881 aatagtgaag tgaaatatca ggtttctggg gataacactg aactgacgtt tacgagttac  
2941 ttgggtattc cacaagaaat caaactctcg ccactccctt ga

**SEQ ID NO. 48: II. Nucleotide sequence for ABCI-nΔ60:**

ttactttacataaaaaactgattgtccccaccgataaagaagcatctaagcatggggacgtcatccacccccgttttctattttgctt  
tacaatgaaaaaccgattgatggttatttactatcgatttcggagaaaaactcatttcaaccagtggaggtcaggcaggctttaagtaa  
aattagatttcactggctggcgtactgtgggagtcctttaataacgatcttgaaaatcgagagatgacctaaatgcaaccaatacctcc  
tctgatggtactcaagacagcattgggcgttcttttaggtgctaaagtcgatagtattcgttttaagcgccttctaattgtgagtcagggtga  
aatctatatcgaccgtattatgttttctgtcgtatgctcgtaccatggctgattatcaagtaaaaactcgttatcagaacctgaaatt  
caatttcacaacgtaaaagccacaactacctgtaacacctgaaaatttagcggccattgatcttattcgccaacgtctaattaatgaattgtc  
ggaggtgaaaaagagacaaacctcgcattagaagagaatatcagcaaaataaaaagtatttcgatgctcttaatactcacactttagca  
aatgggtggaacgcaaggcagacatctgatcactgataaacaaatcattatttatcaaccagagaatcttaactctcaagataaacaactat  
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actaaagcagatgtacttattaatgacaaagcatttattagatcaaggctttgttaaaggagtgctttagtgaacnaccatcactggggat  
acagttctcgttggtggtatattccacgttattaatgtctgatgcactaaagaagcgaacctacaaactcaagtttatgattcattactgtg  
gtattcacgtgagtttaaaagtagtttgatatgaaagtaagtgtgatgctctgatctagattatttcaatacctatctcgcacaacattag  
ccttattactactagacctgatgatcaaaaagcgtatcaacttagttaactttcagccatttatcactggcgcatcaacgcaagtgccac  
cgggtggtaaagatggtttacgccctgatgtacagcatggcgacatgaaggcaactatccgggctactttccagcctttaaaaat  
gcctctcagcttatttatttaccgcgatacaccattttcagtggggtgaaagtgggtggaatagcctgaaaaaagcgtggttcagcgtg  
gatctacagtaatccagaagttggattaccgcttgcaggaagacacctcttaactcaccttcgttaaaatcagtcgctcaaggctattact  
ggcttgccatgtctgcaaaatcatcgcctgataaaacacttgcactctatttattcttgcgattagtataaaacacaaaatgaatcaactgcta  
ttttggagaaactattacaccagcgtctttacctcaaggtttctatgcctttaatggcggtgcttttggtattcatcgttggcagaataaaatg  
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tcaaatagtgtgtaattggctcgcagctttcacagggtatcagcaagaaggttgggattggaatagaatgccaggggcaaccactatc  
caccttctcttaagacttagacagtcctaaacctcataccttaatgcaacgtggagagcgtggatttagcgaacatcatcccttgaag  
gtcaatatggcatgatggcattcgtcttattatcccgcgaatcttgagcggtttgatcctaatttactgcgaaaaagagtgtattagccg  
ctgataatcacttaattttatttggtagcaatataaatagtagtgataaaaaataaaaatgttgaacgaccttattccaacatgccattactcc  
aacattaaataccctttgattaatggacaaaagatagaaaacatgccttatcaaacacacttcaacaagggtgattggttaattgatagc  
aatggcaatggttacttaattactcaagcagaaaaagtaaatgtaagtcgccaacatcaggtttcagcggaaaaataaaaatcgccaacc  
gacagaaggaaactttagctcggcatggatcgtacacagcactcgcaccaagatgccagttatgagtatatggtcttttagatgcgac  
acctgaaaaaatgggagagatggcacaataatccgtgaaaaataatgggttatatcagggttctcgttaaggataaagacgttcatattatt  
ctcgataaactcagcaatgtaacgggatagcctttatcagccagcatcaattgaagacaaatggatcaaaaagggttaataaacctgca  
attgtgatgactcatcgacaaaaagacactcttattgtcagtgagttacacctgatttaaatatgactcgccaaaaagcagcaactcctgt  
caccatcaatgtcacgattaatggcaaatggcaatctgctgataaaaatagtgaaagtgaatatcagggttcttggtgataaactgaactg  
acgtttacgagttactttggtattccacaagaatacaactctcgccactcccttga

**SEQ ID NO. 49: Nucleotide sequence for TAT**

ggctgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgc

**(SEQ ID NO. 50)** Amino acid sequence for a TAT peptide

grkkrrqrrppqc